ESTIMATION OF VITAMIN D LEVEL AND ITS RELATION TO GLYCEMIC CONTROL IN TYPE 2 DIABETES MELLITUS

Dissertation submitted to

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M.D. DEGREE BRANCH - I

GENERAL MEDICINE

GOVERNMENT MOHAN KUMARAMANGALAM

MEDICAL COLLEGE, SALEM

APRIL 2013

CERTIFICATE

This is to certify that the dissertation entitled "**Estimation of Vitamin D level and its relation to glycemic control in type 2 Diabetes Mellitus"** is a bonafide work done by **Dr.S.Arun** in **M.D BRANCH I GENERAL MEDICINE** to be submitted to The Tamil Nadu Dr.M.G.R Medical University, in fulfilment of the University Rules and Regulation for the award of M.D. Degree Branch I General Medicine, under my supervision and guidance, during the academic period from October 2010 to September 2012.

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DECLARATION

I solemnly declare that this dissertation "**Estimation of Vitamin D level and its relation to glycemic control in type 2 Diabetes Mellitus"** was prepared by me at Government Mohan Kumaramangalam Medical College and Hospital, Salem-636030 under the guidance and supervision of **Prof. DR.R. ANBALAGAN, M.D.,** HOD of Medicine, Govt. Mohan Kumaramangalam Medical College and Hospital Salem.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in fulfillment of the University regulations for the award of the degree of M.D. Branch I General Medicine.

Place : Salem

Date :

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INTRODUCTION

Diabetes is a killer disease and a disease of development. Changes in lifestyle and urbanisation combine together to increase a individual's risk for diabetes substantially. In developing countries in particular young people are hit by this epidemic causing significant morbidity and mortality early in their lives. Working people are particularly affected which causes a serious risk to the economical development of these countries. As these people start to live longer and these countries develop this epidemic will only rise alarmingly unless effective treatment and preventive measures are put in place (1).

Vitamin D the so called 'Sunshine Vitamin' is creating interest in the last decade because it has been researched to be associated with a multiple number of diseases like hypertension, heart disease, diabetes ,

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Diabetes is a killer disease and a disease of development. Changes in lifestyle and urbanisation combine together to increase a individual's risk for diabetes substantially. In developing countries in particular young people are hit by this epidemic causing significant morbidity and mortality early in their lives. Working people are particularly affected which causes a serious risk to the economical development of these countries. As these people start to live longer and these countries develop this epidemic will only rise alarmingly unless effective treatment and preventive measures are put in place (1).

Vitamin D the so called 'Sunshine Vitamin' is creating interest in the last decade because it has been researched to be associated with a multiple number of diseases like hypertension, heart disease, diabetes , cancer and so on (2). Research and various interventional studies done on vitamin D uniformly stated that this vitamin is a definite superstar when it comes to maintaining health. The following diseases have been extensively studied and found to be associated with vitamin D deficiency: Hypertension , Obesity, Dyslipidemia , Type 1 and type 2 diabetes mellitus, Coronary artery heart disease, Stroke, Pulmonary tuberculosis, Osteoporosis, Cancer, Rheumatoid arthritis, Multiple sclerosis,

Alzheimer's disease, Depression, Infections, Seasonal affective disorder and overall morbidity and mortality (3).

There is plenty of evidence stating that vitamin D deficiency could well be contributed to the pathogenesis of both types of diabetes (type 1 & 2). The beta cells in the pancreas that is responsible for insulin secretion contains alpha 1 hydroxylase enzyme and vitamin D receptors (VDR's) (4). It has been shown through various interventional studies that vitamin D supplementation improves insulin resistance and glucose tolerance (5) (6). Vitamin D deficiency directly leads to reduced insulin secretion. Indirectly probably by altering the calcium flux through beta cell membranes, it diminish the calcium's ability to influence insulin secretion. In peripheral tissues it enhances insulin action by increasing the expression of insulin receptors, enhances insulin's response to glucose transport, and improving systemic inflammation by decreasing the effects of various cytokines (7).

Studies from all corners of India have universally shown a high prevalence of vitamin D deficiency in our country despite being in the tropical zone. All age groups have been reported to be vitamin D deficient and so are the urban and rural population (8).

Hypertension and diabetes tops the list of fastest growing non communicable diseases worldwide. Currently the prevalence of diabetes in India is very high and if this trend prevails by the year 2030, Indians would be taking the highest toll of the disease worldwide. The reason for glycemic control getting worsened in the winter months is believed to be due to the simultaneous fall of active vitamin D in these seasons (9). Vitamin D3 is inexpensive and readily available. Hence supplementation with the same will help us achieve adequate glycemic control and thereby preventing the dreadful diabetic complications in future. Also the most common associated problems with diabetes like hypertension, cardiovascular diseases, infections can be far lessened.

AIM OF THE STUDY

1) The aim of my present study is to estimate vitamin D levels in patients with type 2 Diabetes and to find out its relation to glycemic control, as assessed by HbA1c percentage.

2) Insulin sensitivity improves with repletion of vitamin D stores as shown in various studies. In my study patient having low vitamin D level and poor glycemic control will be advised vitamin D supplementation accordingly.

LITERATURE REVIEW

Diabetes mellitus is a disease of modern life. It refers to a group of metabolic disorders in man characterized by high blood sugar levels and loss of glucose in the urine. It results from defects in insulin secretion, insulin action or a combination of both. Lack of insulin whether absolute or relative affects the metabolism of carbohydrates, proteins, fats, electrolytes and water with consequences that might be grave. The chronic hyperglycemia associated with this condition causes damage and dysfunction of various organs (kidneys, eyes, nerves, heart and blood vessels) thereby creating widespread morbidity and mortality.

Although a number of specific causes for diabetes mellitus have been identified the etiology and pathogenesis of various forms are far less poorly understood. Most cases of diabetes falls into 2 main pathogenetic categories namely type 1 and type 2 diabetes mellitus (10) . The following image depicts the clinical stages and etiologic types of diabetes which reflects that diabetes progresses through several stages in its natural history and that individual subjects may move from one stage to another in either direction.

90% of cases of diabetes account for type 2 diabetes and is frequently associated with clinical obesity.

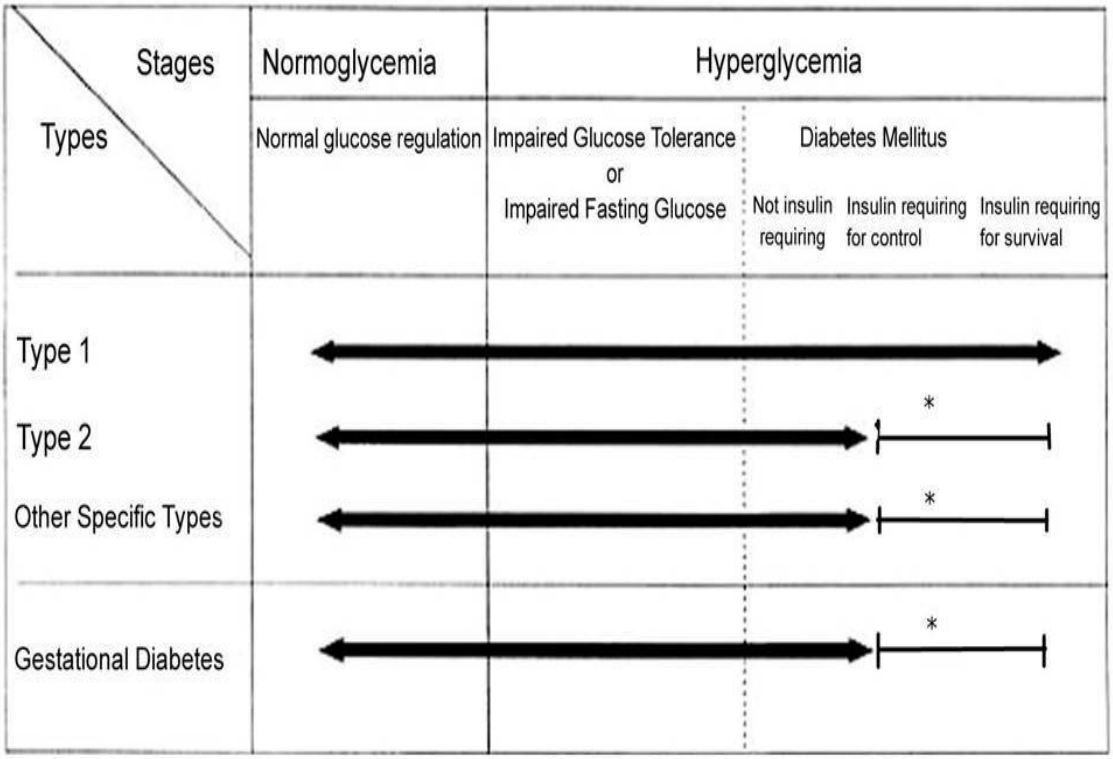


FIG 1 : SHOWING CLINICAL STAGES AND ETIOLOGICAL TYPES OF DIABETES

* In rare instances patient may require insulin

The metabolic derangements associated with diabetes largely reflect the magnitude of the deficiency in insulin concentration and/or insulin action present. Mild deficiency leads to an impairment in the capacity to effectively increase the storage of ingested fuels (eg. Postprandial hyperglycemia) whereas in severe deficiency various

catabolic processess including lipolysis, gluconeogenesis, ketogenesis and proteolysis occurs.

GLOBAL BURDEN :

The number of adults living with diabetes is estimated to be around 366 million people representing 8.3% of global adult population. If this trend prevails the present number will increase to 552 million people or 9.9% of adult population which approximately equals to 3 more people developing diabetes every 10 seconds (11).

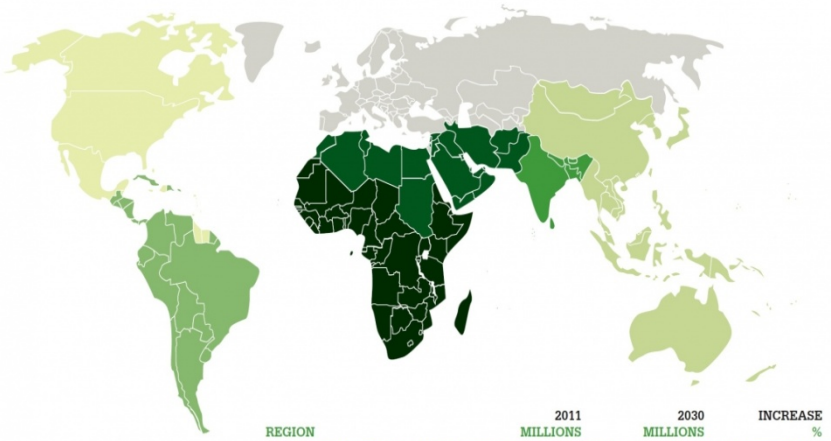
The following statistics are put forward by the International Diabetes Federation (IDF) in their 2012 update (12) :

1) 366 million people have diabetes in 2011, by 2030 this number will increase to 552 million.
2) 80% of people having diabetes live in developing countries.
3) The grunt of the disease are taken by people between 40-59 years of age.
4) The prevalence of type 2 diabetes is increasing in every country.
5) 183 million people (50%) are undiagnosed with diabetes.
6) In 2011, 4.6 million deaths occurred related to diabetes.

7) Incidence of type 1 diabetes is also increasing with 78,000 people developing it every year.

8) Diabetes caused huge economical burden in healthcare expenditures constituting 11% of total health expenditures in adults.

Map: IDF Regions and global projections of the number of people with diabetes (20-79 years), 2011 and 2030



REGION	2011 MILLIONS	2030 MILLIONS	INCREASE %
Africa	14.7	28.0	90%
Middle East and North Africa	32.8	59.7	83%
South-East Asia	71.4	120.9	69%
South and Central America	25.1	39.9	59%
Western Pacific	131.9	187.9	42%
North America and Caribbean	37.7	51.2	36%
Europe	52.6	64.0	22%
World	**366.2**	**551.8**	**51%**

Top 10: Countries/territories of number of people with diabetes (20-79 years), 2011 and 2030

	COUNTRY /TERRITORY	2011 MILLIONS		COUNTRY /TERRITORY	2030 MILLIONS
1	China	90.0	1	China	129.7
2	India	61.3	2	India	101.2
3	United States of America	23.7	3	United States of America	29.6
4	Russian Federation	12.6	4	Brazil	19.6
5	Brazil	12.4	5	Bangladesh	16.8
6	Japan	10.7	6	Mexico	16.4
7	Mexico	10.3	7	Russian Federation	14.1
8	Bangladesh	8.4	8	Egypt	12.4
9	Egypt	7.3	9	Indonesia	11.8
10	Indonesia	7.3	10	Pakistan	11.4

(Images from IDF Diabetes atlas fifth edition 2012 Update)

Diabetes at a glance, 2012 South-East Asia (SEA) (13) :

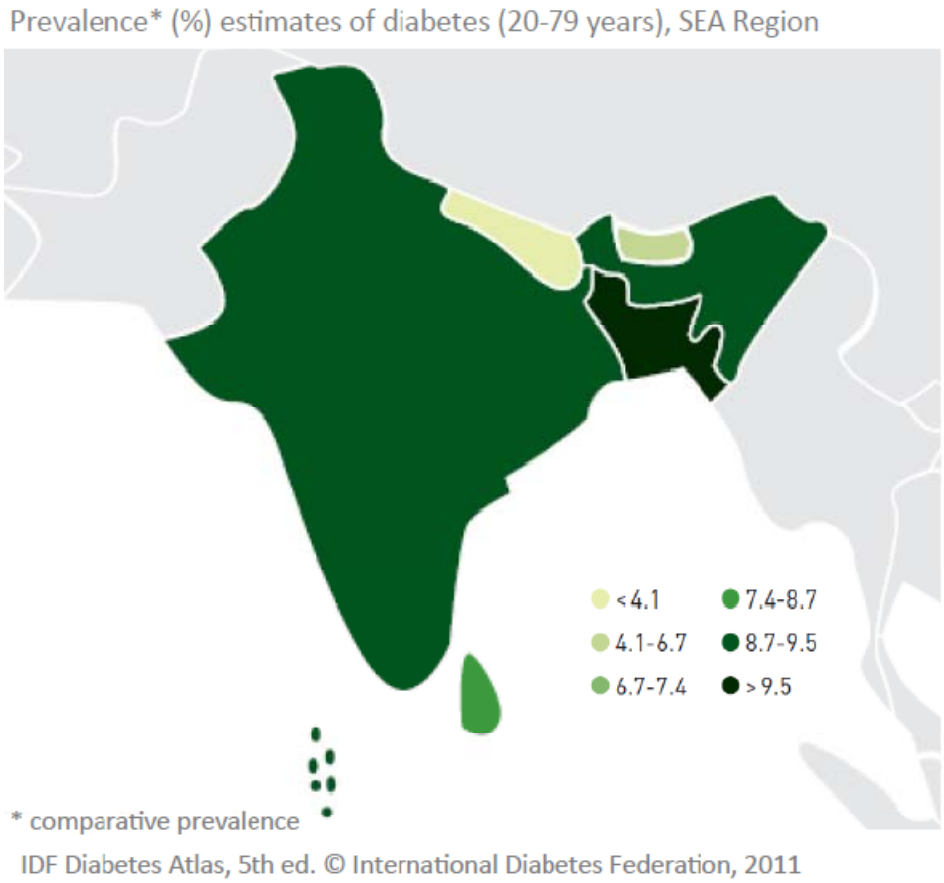
Larger than **70.3 million** people in the South east asia Region have diabetes; by 2030 this number will probably rise to **120.9 million.**

1) **8.7 % adults** in SEA Region have diabetes.

2) **One fifth** of all adult diabetic patients, live in this region .

3) **1.1 million deaths has been caused by diabetes** in the SEA region this year.

4) USD **4.6 billion** were spent on treating diabetes in the region.



Over 30 million people in India has been diagnosed with diabetes. The crude prevalence rate in urban areas of India is about 9%. In rural areas the prevalence is approximately 3% of the total population. Various studies in India have shown that Indian diabetic subjects have very poor glycemic control (HbA1c > 8%). Diabetes is also beginning to appear much earlier in life in India. Also the closely associated problems like Hypertension, Dyslipidemia, Obesity, Coronary artery heart disease and Peripheral vascular disease are in very percentage (14) (15). Overall the management of diabetes and care in India is overwhelming and leaves much less to be desired. Increased awareness among health care professionals to improve the standards of diabetes care in India is the need of the hour.

VITAMIN D – ‘THE SUNSHINE VITAMIN’ :

Vitamin D is a fat soluble steroid vitamin with multitude of functions in the body. In nature only a few foods contain vitamin D such as fatty fish, liver and egg yokes (16). However most foods are fortified with vitamin D most commonly milk and breakfast cereal. Most of the vit D formed in the body is due to the result of sun exposure on the skin.

An adult in a bathing suit exposed to one minimal erythemal dose of ultraviolet radiation (a slight pinkness of the skin after exposure) is equivalent to ingesting 10,000 – 25000 IU of vitamin D. A variety of factors limit the skin’s production of vitamin D3 like aging, use of sunscreens and increased skin pigmentation.

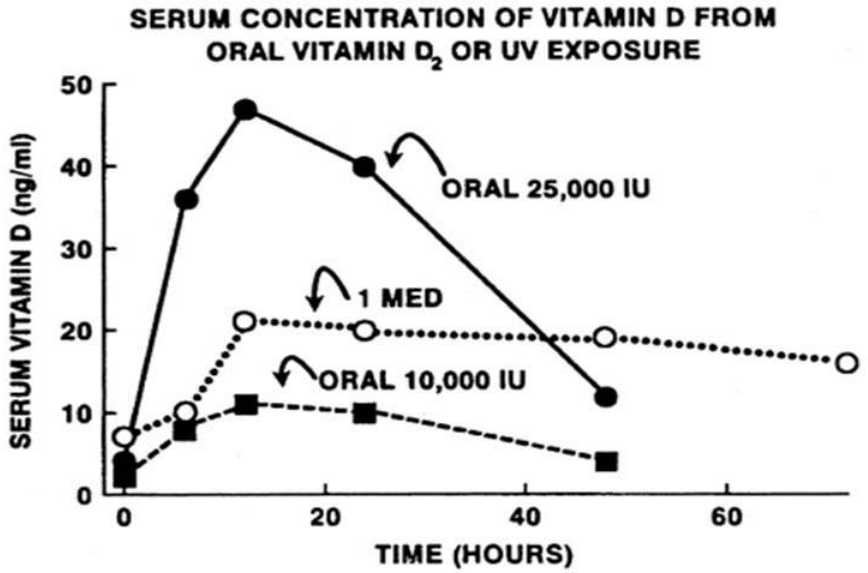


Figure showing Comparison of vitamin D3 levels after a whole body exposure to 1 MED (minimal erythemal dose) of sunlight compared with a single oral dose of either 10,000 0r 25000 IU of vit D.

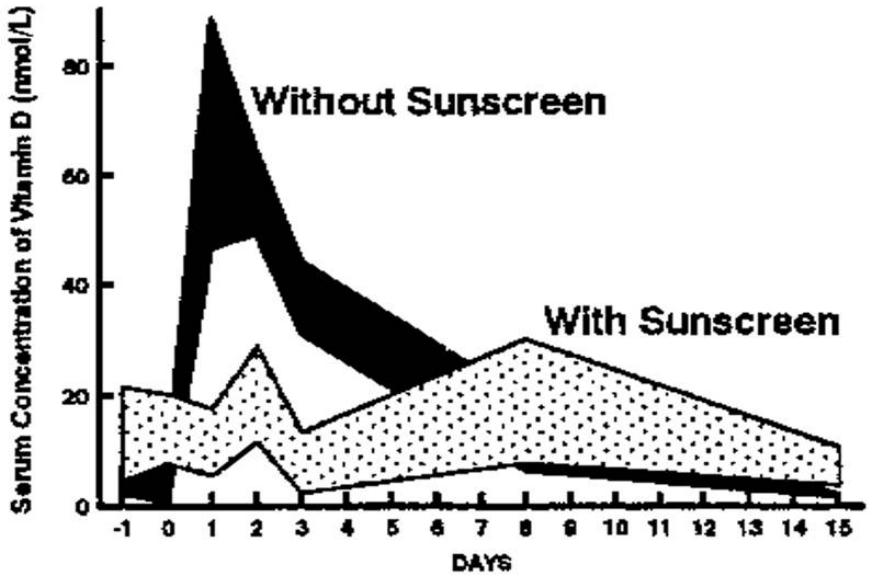


Figure showing the effect of sunscreens in decreasing the production of vitamin D and thereby serum concentrations.

Suggested dietary sources are listed below (17):

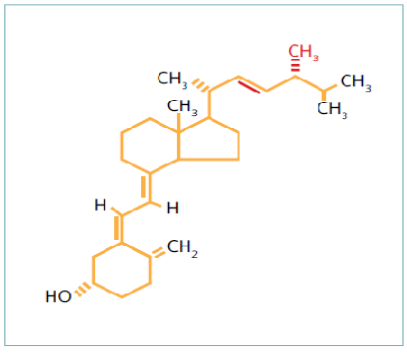
Selected Food Sources of Vitamin D

Food	International Units	Daily Value, %*
Cod liver oil, 1 tablespoon	1360	340
Salmon, cooked, 3 ½ ounces	360	90
Mackerel, cooked, 3 ½ ounces	345	90
Sardines, canned in oil, drained, 3 ½ ounces	270	70
Eel, cooked, 3 ½ ounces	200	50
Milk, nonfat, reduced fat, and whole, vitamin D fortified, 1 cup	98	25
Margarine, fortified, 1 tablespoon	60	15
Cereal grain bars, fortified with 10% of the DV, 1 each	50	10
Pudding, ½ cup prepared from mix and made with vitamin D fortified milk	50	10
Dry cereal, vitamin D fortified with 10% of DV, ¾ cup (other cereals may be fortified with more or less vitamin D)	40-50	10
Liver, beef, cooked, 3 ½ ounces	30	8
Egg, 1 whole (vitamin D is present in the yolk)	25	6

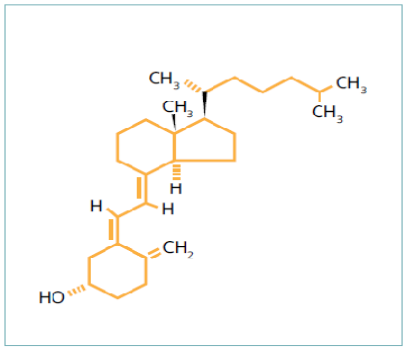
DAILY VALUE (DV) expressed in % are reference values developed by FDA to help consumers determine if a food contains a little or lot of a particular nutrient. The DV for vit D is 400 IU for adults.

There are five forms of vitamin D (Vit D1, D2, D3, D4, D5) of which vitamin D2 and D3 are the major forms in human beings. They are collectively called as ‘calciferol’(18).

Vitamin D_2 (Ergocalciferol)

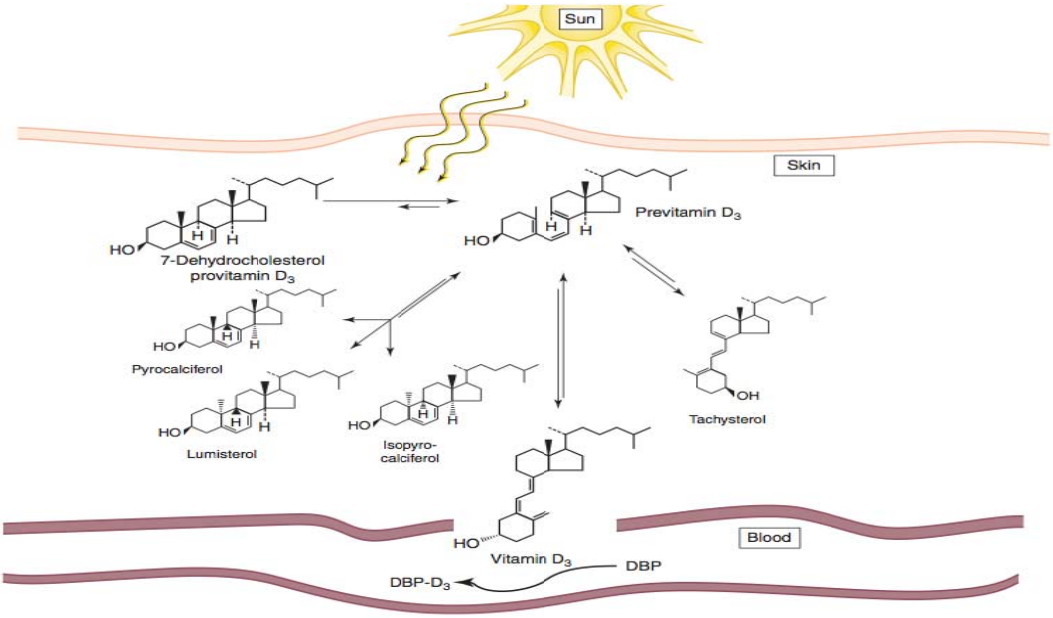


Vitamin D_3 (Cholecalciferol)



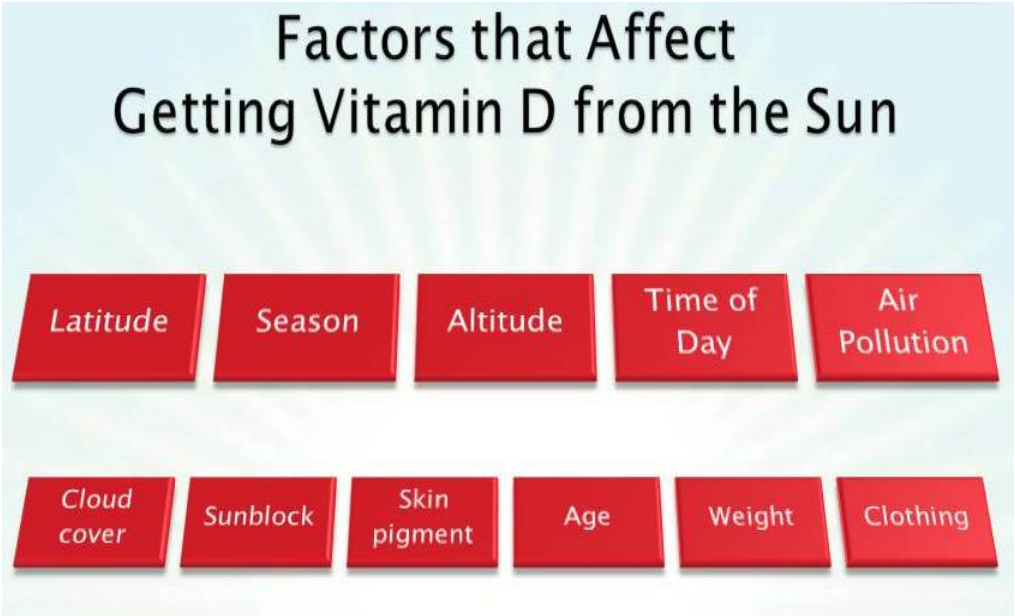
PRODUCTION AND METABOLISM :

Most of the vitamin D needed by the body is generated in the skin by the production of cholecalciferol. Vitamin D3 is produced in the skin from 7 dehydrocholesterol after exposure to sun in a series of biochemical reactions. This production is dependent on the specific ultraviolet (UV) wavelength of sun usually between 290-315 nm and the total number of photons absorbed by 7 dehydrocholesterol in the skin.

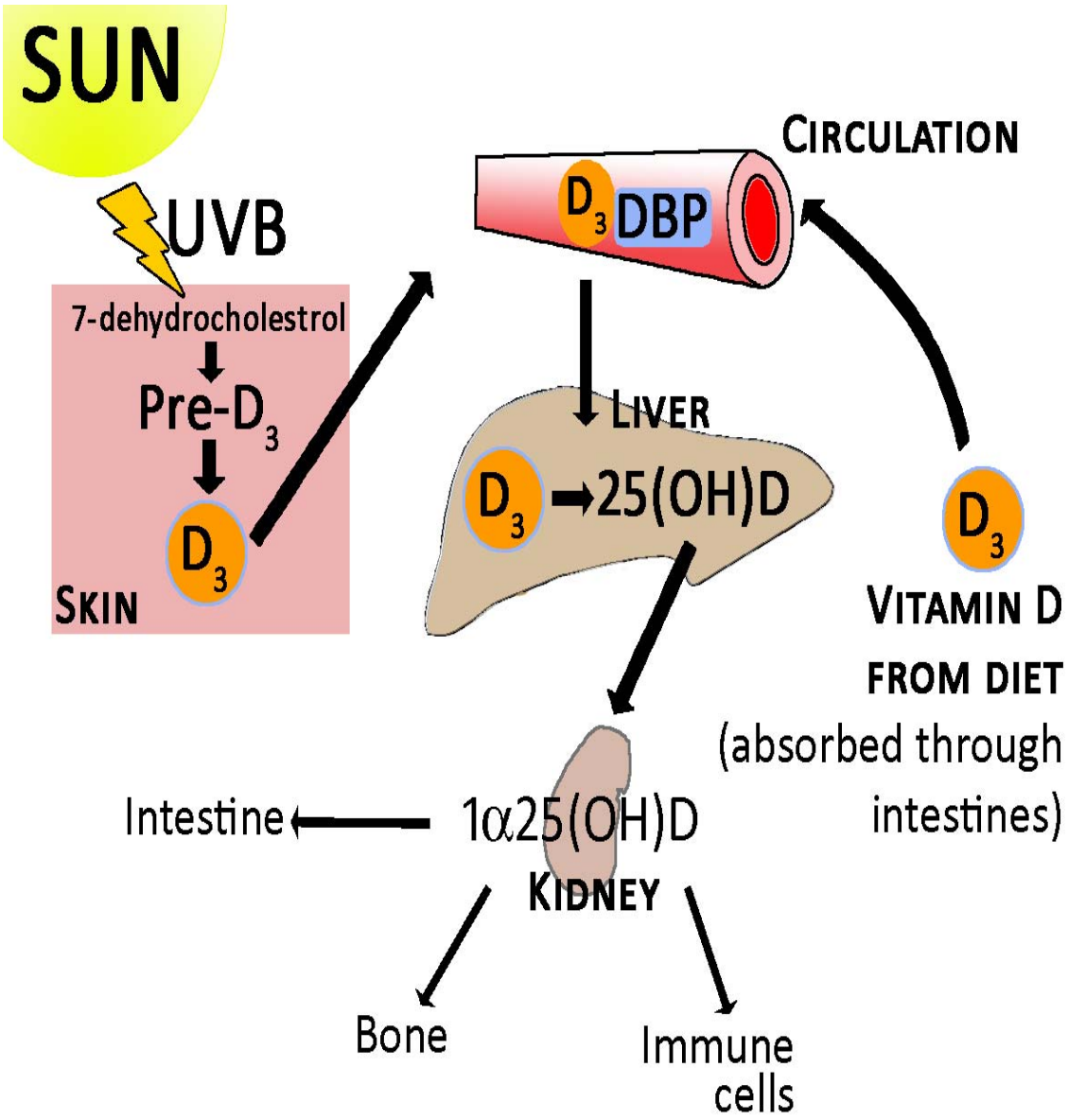


A variety of factors affect this metabolic process like use of sunscreen lotions, increased skin pigmentation, and more importantly ‘Zenith Angle’ (19), which is defined as the angle at which the sunlight reaches the surface of earth which is again depends on the specific time of the day, season and appropriate latitude. The UV radiation required to

produce vitamin D is blocked by the earth's atmosphere when the sun did not rise to over 35° above the horizon (20). Thus synthesis of vitamin D will be poor in the two extremes of the day (morning & evening) , in the winter months and more so in areas of higher latitude(21) (22).



After vitamin D3 is produced whether in the skin or consumed in the food , it enters the circulation and metabolised in the liver by 25 hydroxylase (23) to 25 Hydroxy vitamin D (25(OH)D) which is the major circulating and storage form of vitamin D. 25(OH)D is then converted to 1,25 Dihydoxy vitamin D (1,25 $(OH)_2D$) , the active hormonal form of vitamin by the enzyme 1,25 alpha hydroxylase (24) in the kidney. The active vitamin is then transferred to the target tissues after binding to vitamin D binding protein (DBP) (25).



MOLECULAR ACTIONS OF VITAMIN D :

At the cellular level there are two types of responses to $1,25(OH)_2$ D : the rapid response which occurs within seconds to minutes and the genomic response which occurs from minutes to days (26). In the rapid response the active vitamin D binds to either vitamin D receptor (VDR)

or the vitamin D membrane associated rapid response steroid binding protein (MARRS) in the plasma membrane. This activates the G protein that causes increased intracellular cyclic AMP which increases the activity of protein kinase C and mitogen activated protein kinase leading to biologic responses (27). During the genomic response the $1,25(OH)_2D$ binds to nuclear VDR and dimerizes with retinoic X receptor (RXR) forming a heterodimer which binds to vitamin D responsive elements (VDRE) modulating tissue specific gene expressions and protein synthesis (28) (29). Ultimately this genomic effect will lead to physiological responses.

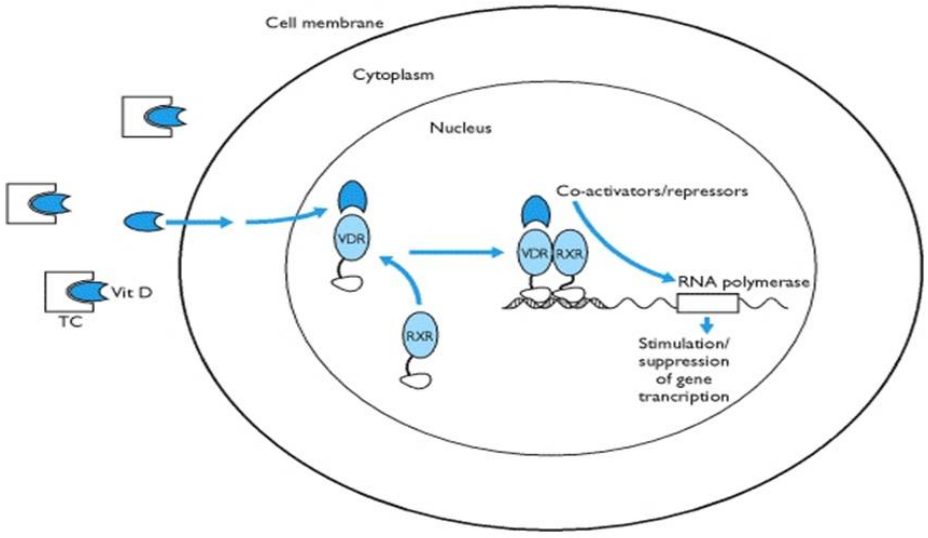
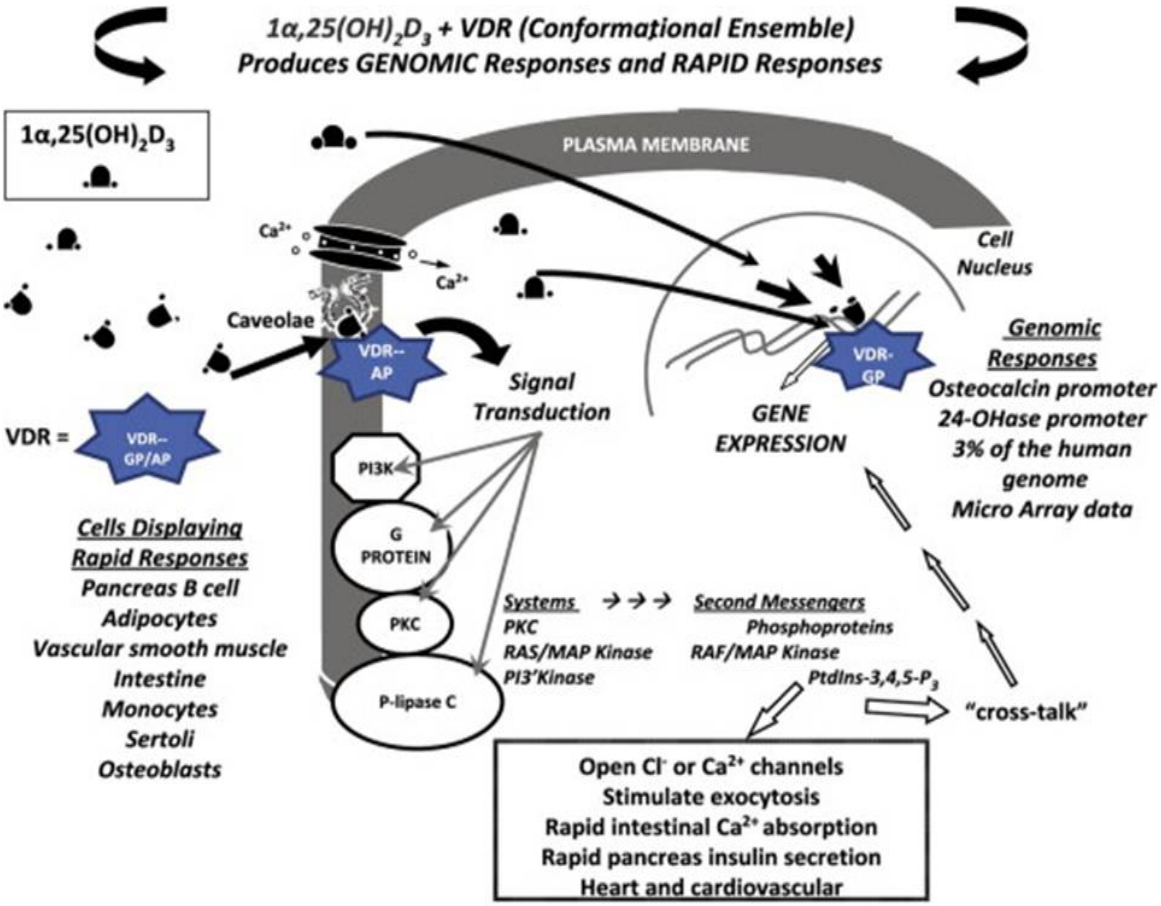


Figure showing interaction of vitamin D receptor(VDR) with retinoic X receptor in regulating gene expression.



Rapid & Genomic responses of vitamin D: $1\alpha,25(OH)_2D_3$ can interact with the VDR localized in the cell nucleus to generate genomic responses via regulation of gene transcription. Binding of $1\alpha,25(OH)_2D_3$ to the caveolae-associated VDR may result in the activation of one or more second messenger systems(phospholipase,protein kinase c) resulting in rapid responses.

BIOLOGIC FUNCTIONS OF VITAMIN D:

1) The primary function of vitamin D is to promote the absorption of calcium in the intestine and therby maintaining adequate serum calcium (Ca^{2+}) and phosphate (Po_4^{2-}) concentrations. This enables normal mineralization of bone. It is also required for bone growth and remodelling by osteoblasts & osteoclasts (30).

2) Despite normal serum calcium levels the parathyroid gland has a tendency to hyperproliferate if vitamin D is deficient. Thus Vitamin D helps to suppress the parathyroids by a negative feedback mechanism, which is a essential part in the treatment of renal osteodystrophy (31).

3) Vitamin D may enhance immune system function and reduce the risk of autoimmunity, a condition in which the body attacks itself by production of various auto antibodies. It decreases the expression of various cytokines thereby reducing systemic inflammation. Chronic inflammation causes blood vessel damage and dyslipidemia and can increase the risk of developing heart disease. By this function vit D gives protection against various auto immune disease and coronary artery heart disease.

4) Vit D plays a role in insulin secretion in pancreatic beta cells and improves insulin sensitivity in peripheral tissues. Thus plays a crucial role in the pathogenesis of both type 1 and type 2 diabetes mellitus.

5) Prevention of cancer especially prostate, colon and breast cancer(32).

6) Neuroprotection (33).

7) Prevention of infections especially pulmonary tuberculosis, and prevention of exacerbation in asthmatics.(34).

8) Vitamin D has various renal protective effects. They include attenuation of the development of glomerulosclerosis and retarding the progression of albuminuria thereby preventing progression to chronic kidney disease (35).

9) Alteration in vit D status causes increased propensity to become obese by upregulating insig 2 (insulin induced gene 2) gene expression in the adipose tissue that causes fat mass accumulation(36).

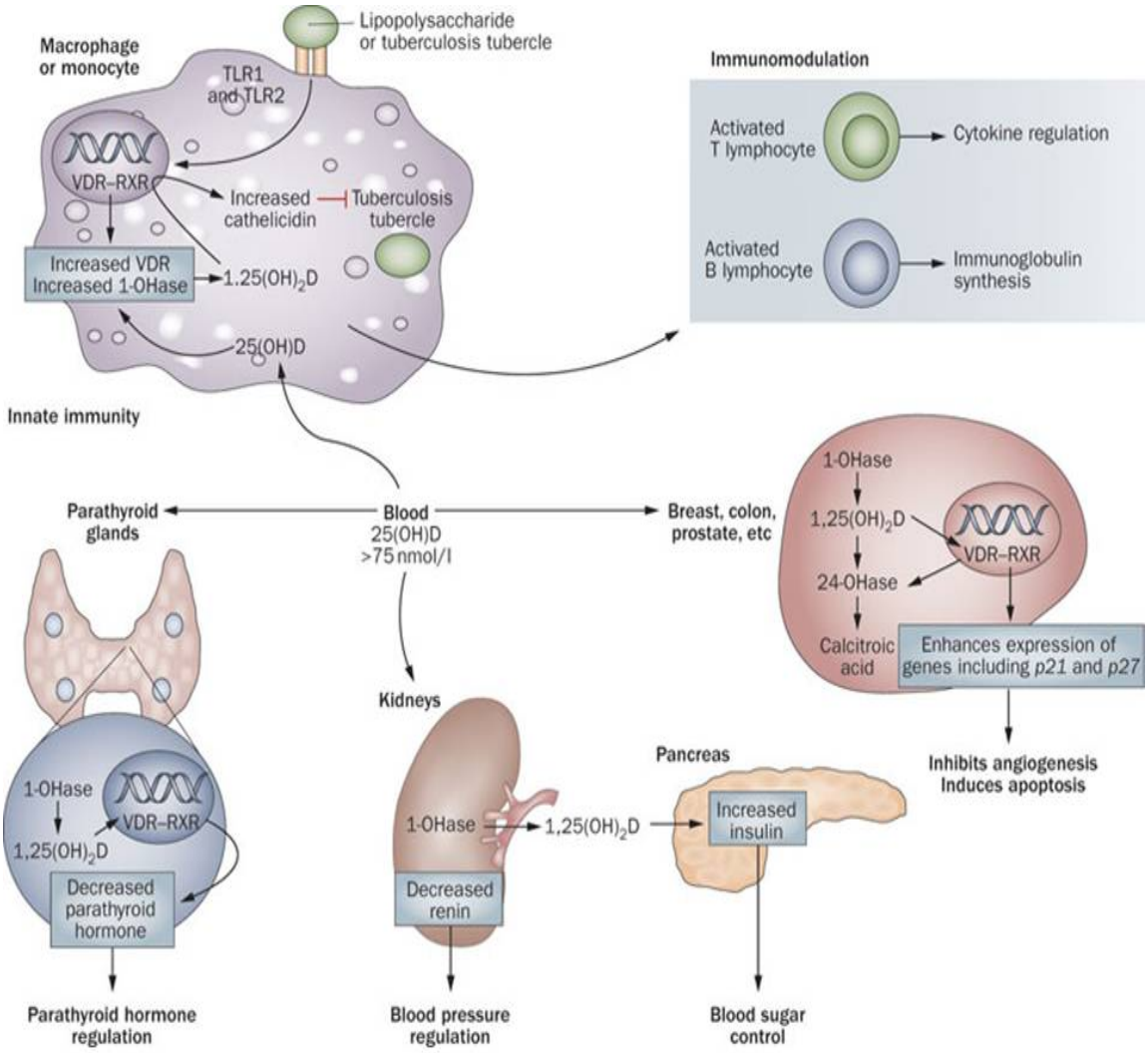
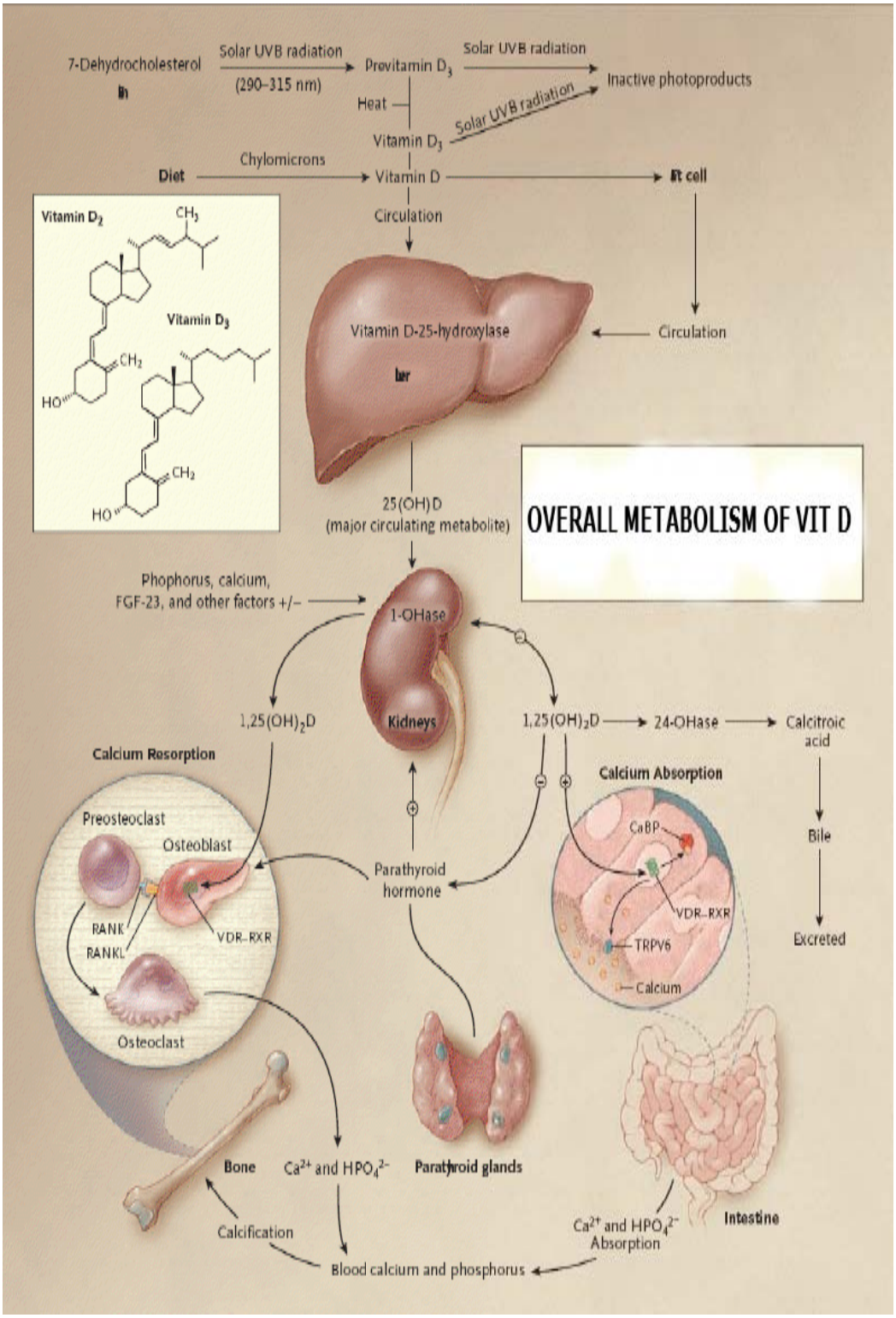


Figure showing major biological functions of vitamin D

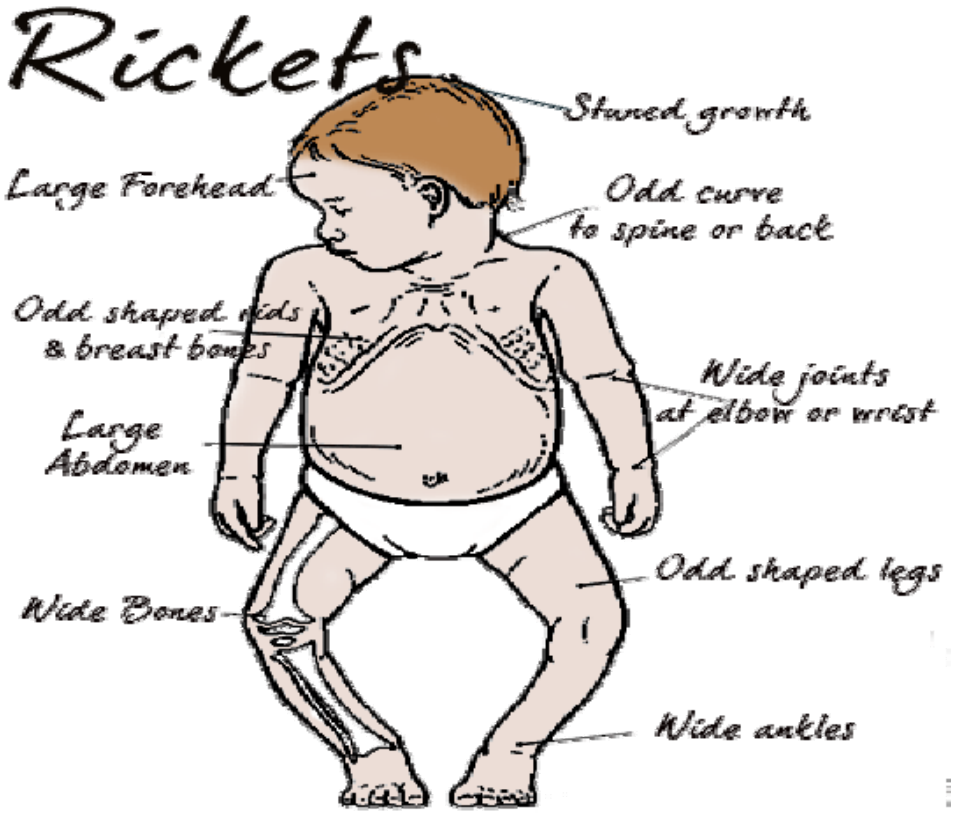
OVERALL METABOLISM OF VITAMIN D:



Vitamin D Deficiency:

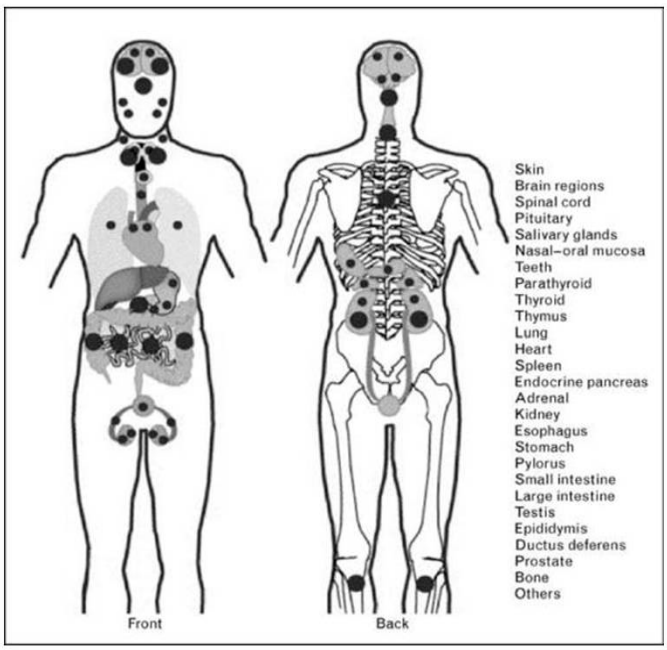
The symptoms of vitamin D deficiency are those of calcium deficiency. The bones fail to calcify normally and may grow so weak that it cannot support the weight of the body developing the characteristic bow legs, the most obvious sign of the disease in children with ‘Rickets’. The adult form of rickets is called ‘Osteomalacia’.

Inadequate vitamin D is a recognised risk factor for Osteoporosis (reduced mineral density). Without vitamin D, the absorption of calcium is limited and bone remodelling is impaired. This combination leads to a loss of bone mass.



Changing face of Vitamin D :

Over the past decade it was realised that vitamin D deficiency is no longer just about bones. After the understanding that vitamin D receptors has been extensively distributed in various tissues of the body like skin keratinocytes, almost all cells regulating immune system, pancreatic islet cells, enterocytes, proximal renal tubular cells, cancer cells such as colon, breast, prostate and lung, hair follicular cells, testis, prostate, ovaries, granulomatous tissue in sarcoidosis, smmoth muscles, the face of vitamin D has changed. Soon various autoimmune diseases and many cancers has been studied and found to be associated with vitamin D deficiency.



Target sites of vitamin D - 'Vitamin D receptor homunculus'

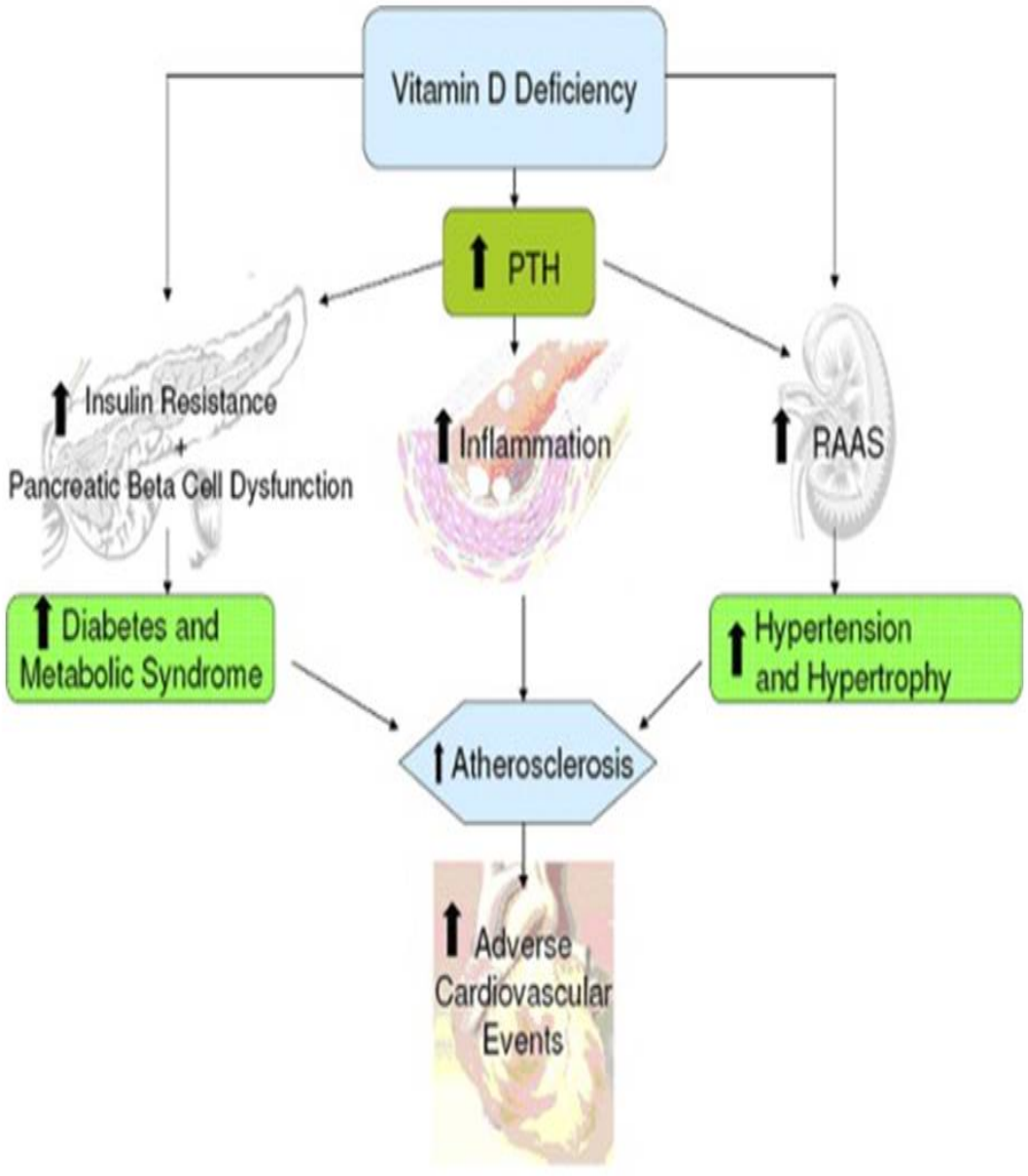
The following diseases has been linked to vitamin D deficiency (37):

- Osteoporosis / osteomalacia
- Coronary artery heart disease
- Stroke
- Hypertension
- Arteriosclerosis
- Cancer especially prostate, breast, colon, lung and non Hodgkin's lymphoma
- Chronic muscle, bone and joint pain
- Dental caries,
- Periodontal disease.
- Metabolic syndrome.
- Type 1 and type 2 diabetes mellitus
- Obesity
- Inflammatory bowel diseases

- Rheumatoid arthritis
- Sjogren’s syndrome.
- Various autoimmune diseases (multiple sclerosis)
- Pulmonary tuberculosis
- Epilepsy
- Alzheimers disease
- Parkinson’s disease.
- Schizophrenia ,
- Depression and mood disorders
- Chronic fatigue syndrome.
- Autism
- Attention deficit hyperactivity disorder (ADHD).
- Infertility
- Bronchial asthma and upper respiratory tract infections.
- Preeclampsia

- Congestive heart failure
- Peripheral vascular disease.
- Premature greying of hair.
- Osteoarthritis.
- Fibromyalgia
- Muscle myopathy and increased falls.
- Psoriasis.
- Congenital birth defects.

Figure demonstrating vitamin D deficiency causing adverse cardiovascular events



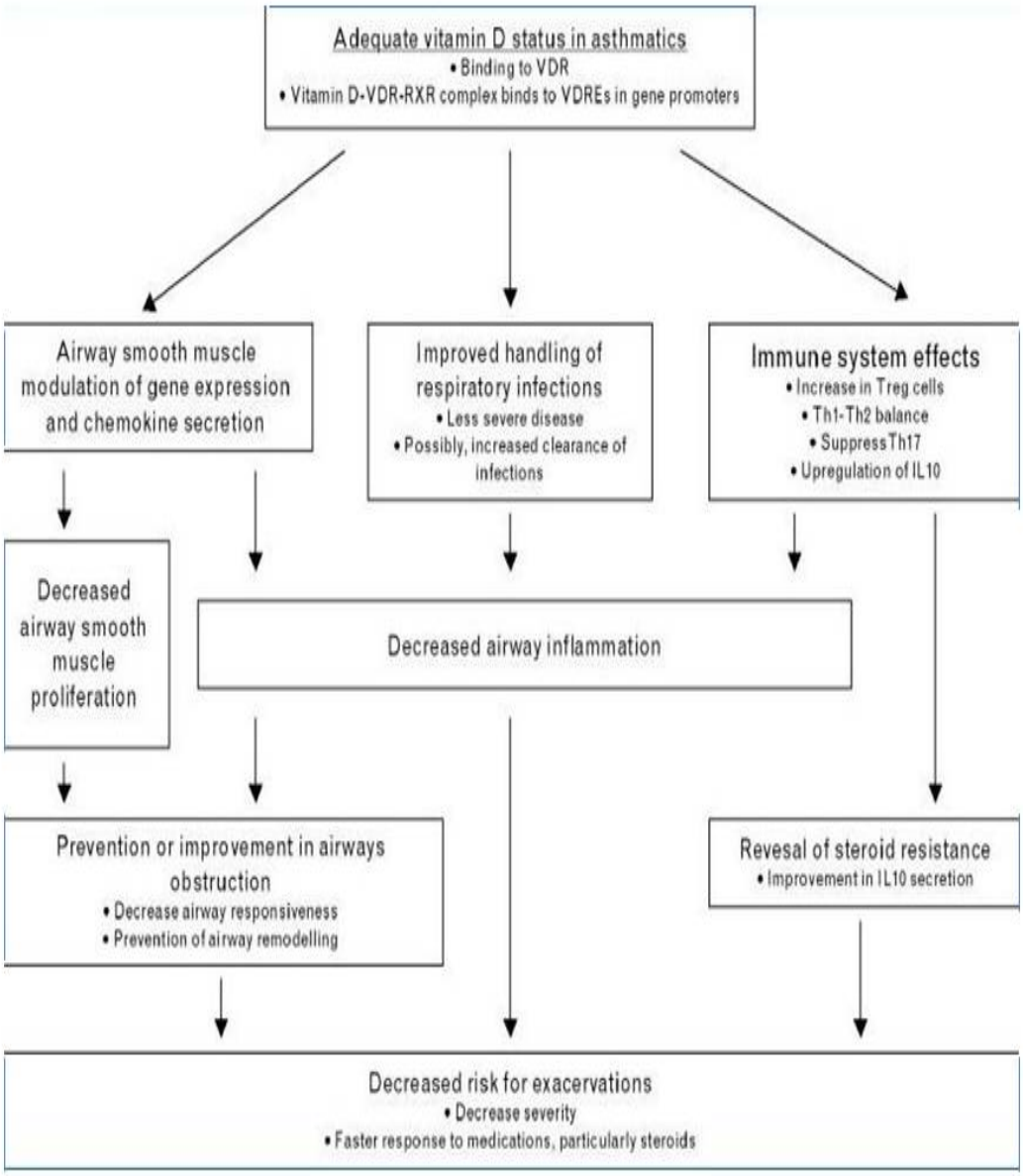


Figure showing importance of vitamin D in asthmatics

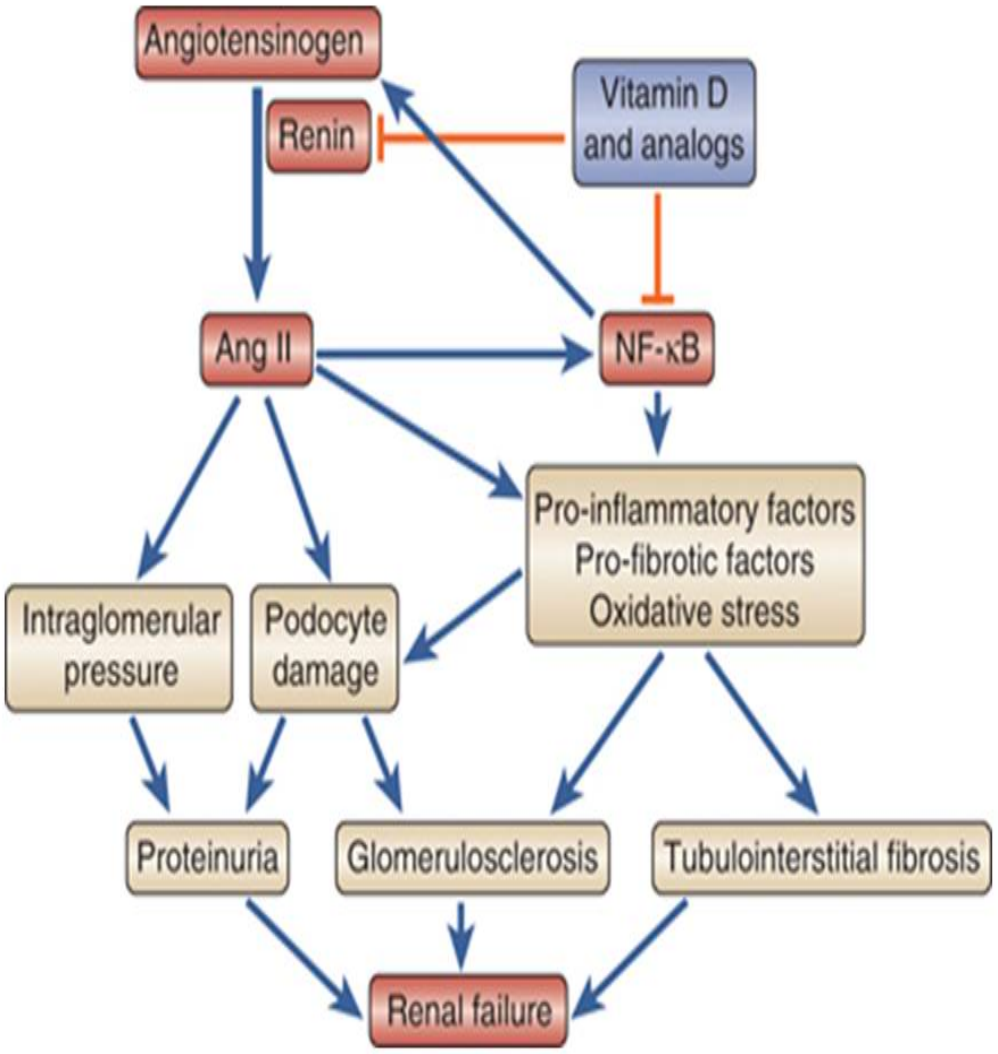
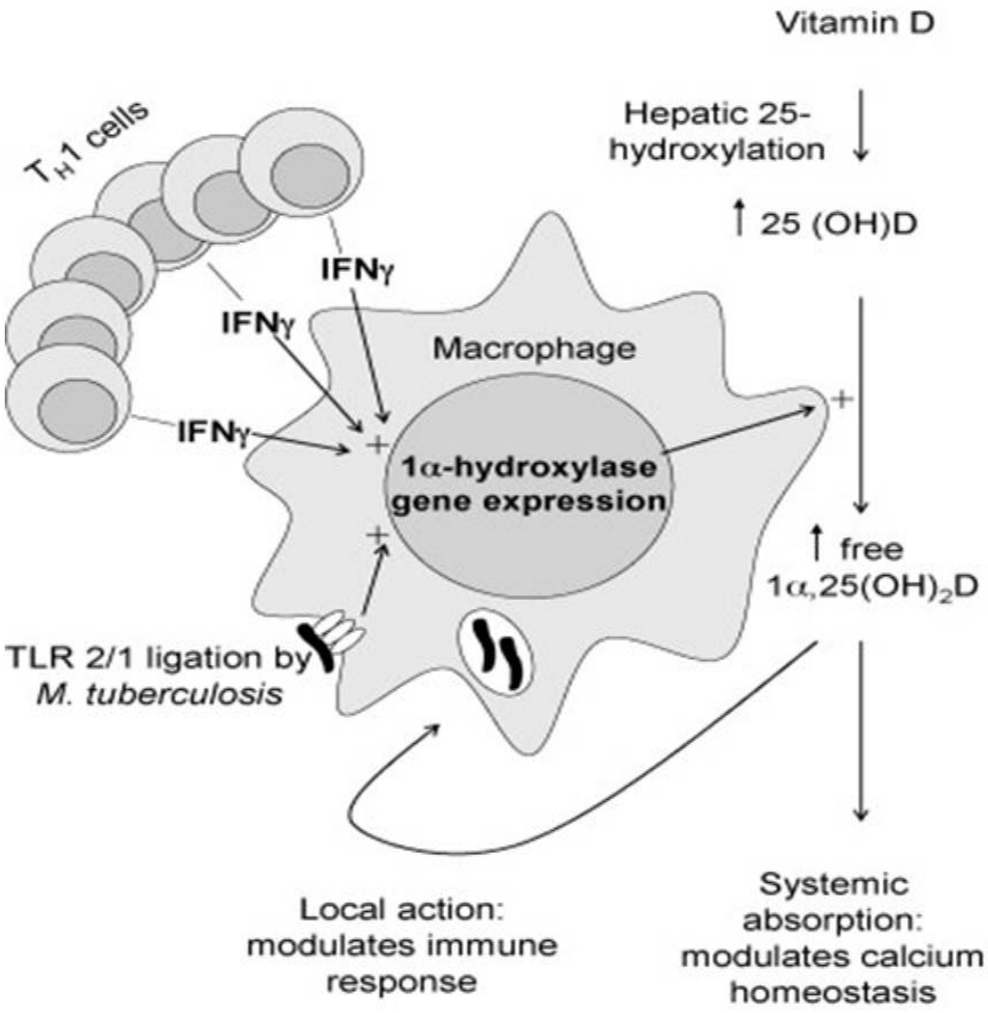
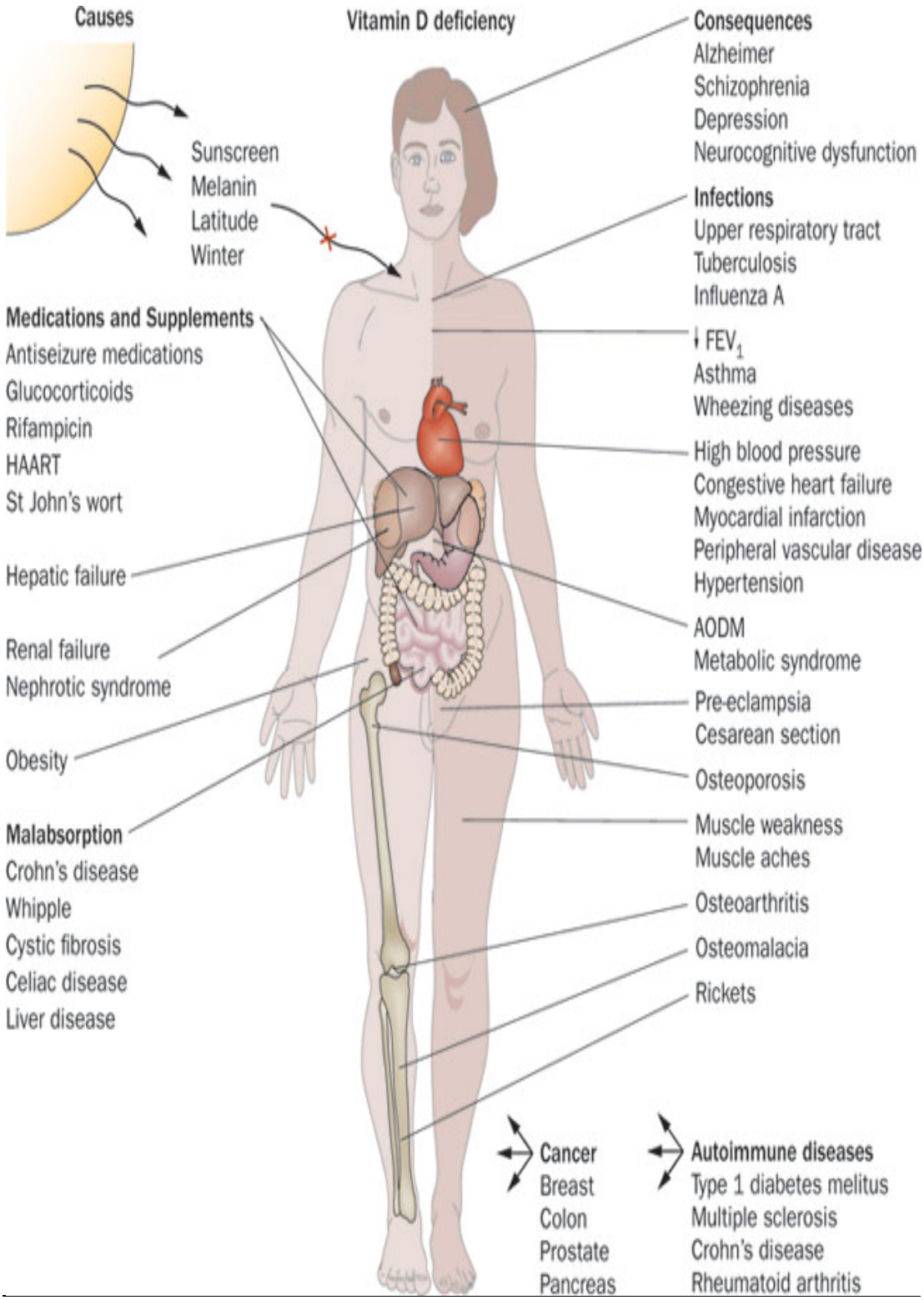


Figure showing the renoprotective effects of vitamin D

The following image shows the role of vitamin D as an adjunct to Anti tuberculous therapy (ATT) in pulmonary tuberculosis.



In the granuloma both IFNγ and ligation of macrophage TLR2/1 by *M. tuberculosis* induces macrophage expression of 25(OH)D-1α-hydroxylase. Vitamin D supplementation results in increased circulating concentrations of free 25(OH)D, which is metabolised by upregulated 1α-hydroxylase to 1α,25$(OH)_2$D. This may either act in a paracrine manner to modulate immune responses in the granuloma.


Causes
Vitamin D deficiency
Consequences
Alzheimer
Schizophrenia
Depression
Neurocognitive dysfunction
Sunscreen
Melanin
Latitude
Winter
Infections
Upper respiratory tract
Tuberculosis
Influenza A
Medications and Supplements
Antiseizure medications
Glucocorticoids
Rifampicin
HAART
St John's wort
↓ FEV1
Asthma
Wheezing diseases
High blood pressure
Congestive heart failure
Myocardial infarction
Peripheral vascular disease
Hypertension
Hepatic failure
Renal failure
Nephrotic syndrome
AODM
Metabolic syndrome
Pre-eclampsia
Cesarean section
Obesity
Osteoporosis
Malabsorption
Crohn's disease
Whipple
Cystic fibrosis
Celiac disease
Liver disease
Muscle weakness
Muscle aches
Osteoarthritis
Osteomalacia
Rickets
Cancer
Breast
Colon
Prostate
Pancreas
Autoimmune diseases
Type 1 diabetes melitus
Multiple sclerosis
Crohn's disease
Rheumatoid arthritis

VITAMIN D AND DIABETES MELLITUS:

Vitamin D deficiency has shown to be associated with both type 1 and type 2 diabetes mellitus.

Vit D and Type 1 diabetes mellitus :

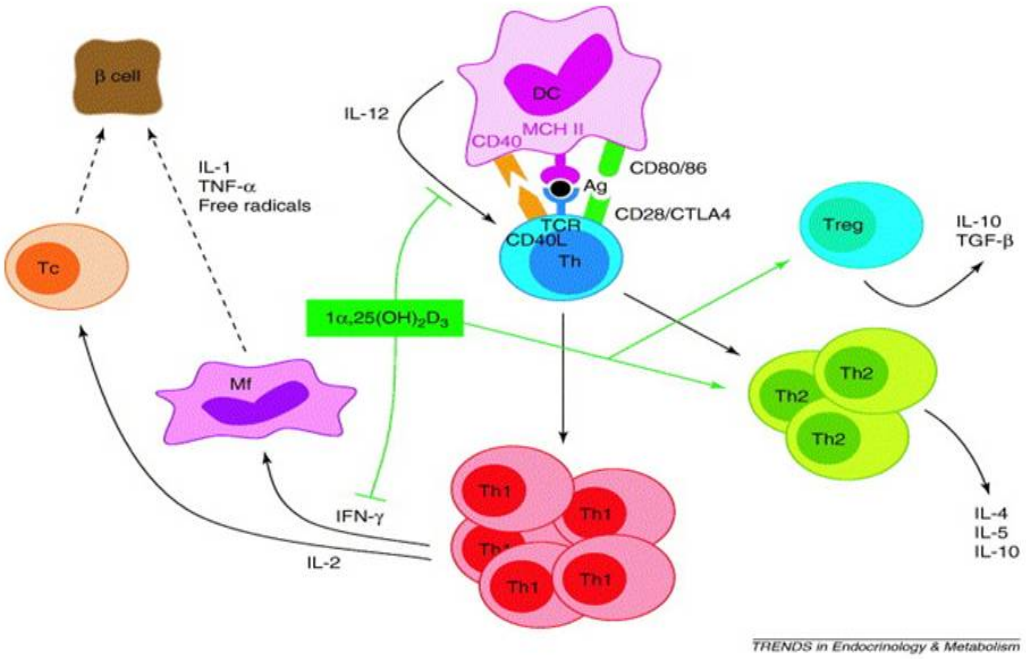
Autoimmune destruction of pancreatic beta cells is the pathogenetic mechanism causing type 1 diabetes. Almost all cells regulating the immune system contain Vitamin D receptors (VDR) (38).

1. Vitamin D inhibits dendritic cell maturation, repress type 1 cytokines, and upregulate regulatory T cells (39).

2. Vitamin D also suppresses the antigen presenting capacity of macrophages, modulates CD4 Lymphocyte development and inhibit the production of interferon c and interleukin2. These cytokines have a role in activating macrophages and cytotoxic T Lymphocytes which in turn lead to beta cell destruction that secretes insulin.

3. Thus by its immunomodulatory action (40) vitamin D plays a role in the pathogenesis of type 1 diabetes.

In recent onset type 1 DM, administration of 0.25 µg calcitriol on alternate days for 1 year has been shown to have a modest effect on residual pancreatic beta cell function after 6 months suggesting a potential role in treatment after diagnosis.



IMMUNOMODULATORY EFFECTS OF VIT D

-Inhibits the surface expression of MHC class II complexed antigen.

-Inhibits the production of Th1 cytokines and stimulates Th2 cytokines.

-Favours induction of regulatory T cells (Th2 and Tregs) which inhibit Th1 cells through production of inhibitory cytokines. These effects lead to protection of target tissues like beta cells in type 1 DM, Autoimmune diseases and transplantation.

VITAMIN D AND TYPE 2 DIABETES :

The pathogenesis of type 2 diabetes involves both beta cell dysfunction and insulin resistance. However insulin resistance is the major culprit which is defined as the common pathologic state in which target cells (muscle, liver and adipose tissue) fails to respond to normal levels of circulating insulin (41).

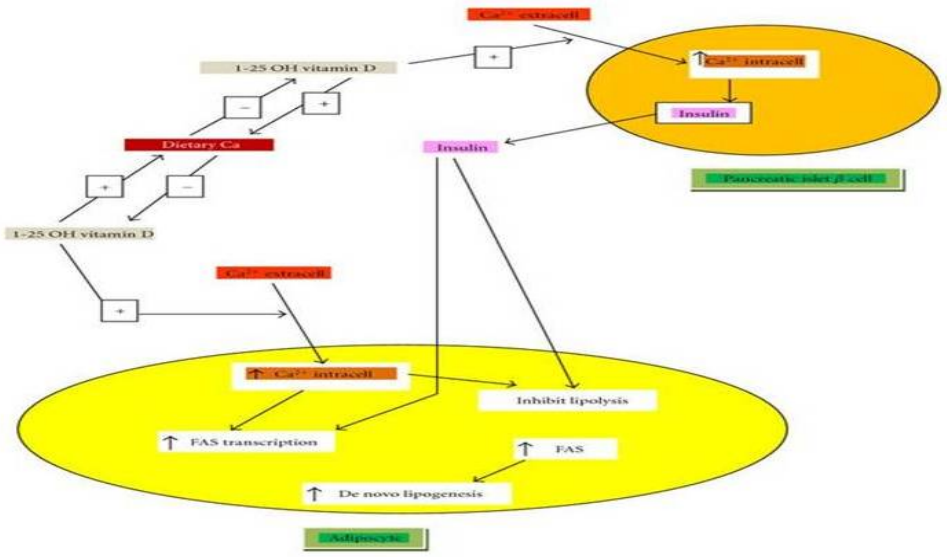
The following are the mechanisms by which vitamin D deficiency has been linked to insulin resistance and type 2 diabetes:

- Optimal concentrations of intracellular calcium (140-370nm) is needed for insulin to mediate its effect on target tissues. Vitamin D deficiency leads to elevated parathyroid hormone levels (PTH). This hyperparathyroidism cause a paradoxical rise in intracellular calcium level (‘calcium paradox’) more than the optimal level that results in diminished cellular response to insulin (42).

- Vitamin D regulates the transcription & expression of insulin receptors in peripheral tissues thereby enhancing insulin responsiveness for glucose transport (43).

- Vitamin D decreases the expression of various inflammatory cytokines thereby keeping systemic inflammation in check (44).

- The mechanism of insulin secretion in beta cells is critically dependent on changes in intracellular calcium concentration. Vitamin D regulates extracellular calcium levels and calcium flux through beta cell membranes. Either vitamin D or calcium deficiency alter the physiological balance between intracellular and extracellular calcium in beta cells interfering with insulin synthesis and secretion (45).



Dietary and intracellular calcium + vit D modulation of adiposity

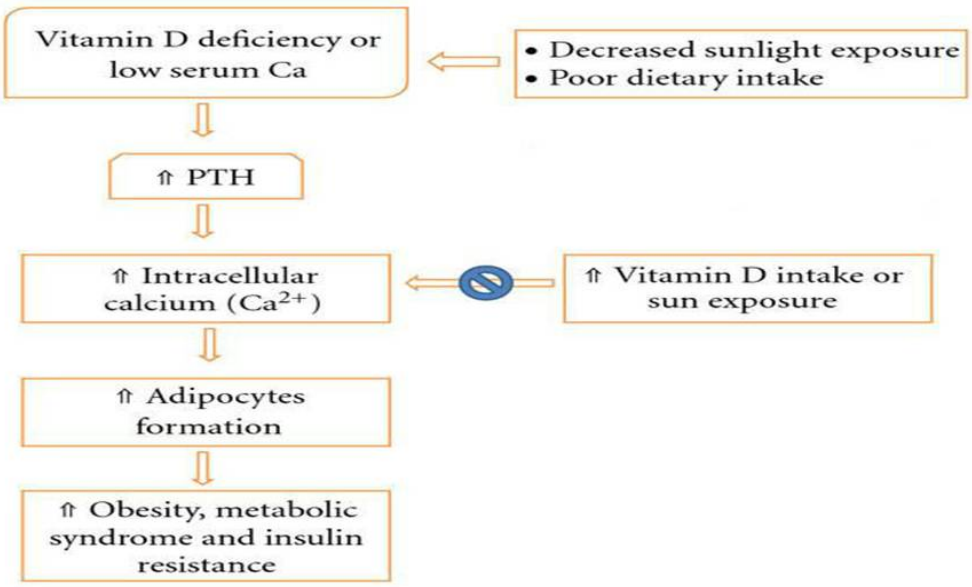
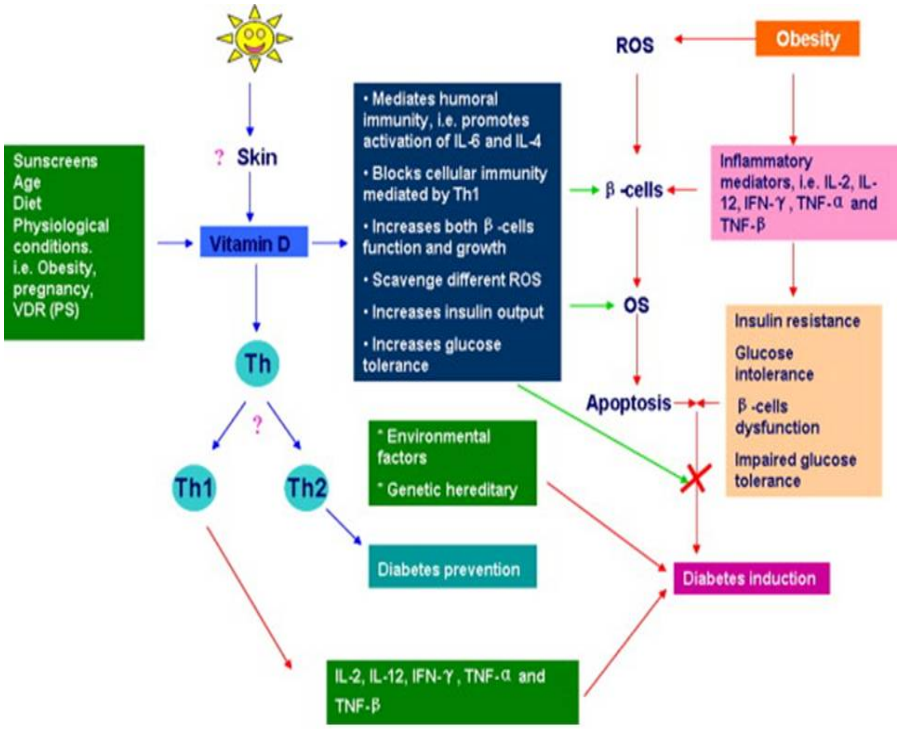


Figure showing relationship between Vit D, PTH, Calcium and in causing insulin resistance.



The role of the different pathogenetic effectors in the etiology of diabetes, and the possible mechanisms through which vitamin D might prevent diabetes.

HUMAN STUDIES LINKING VITAMIN D AND DIABETES :

- The NHANES (National Health And Nutrition Examination Survey) Group (46) :

  Study period : 2003-2006. Evaluated 9,773 U.S. adults > 18 years.

  RESULT : Study showed a mechanistic link between serum vitamin D levels, glucose homeostasis and the evolution of diabetes.

- Kositsawat et al : (47)

  RESULT : Based on their own study concluded that patients with elevated A1C levels should be evaluated for vitamin D insufficiency (47).

- Kayaniyil et al : (49)

  - A linear regression analysis of 712 subjects after evaluating serum 25(OH)D levels and assessing insulin sensitivity by means of the homeostaSis model of insulin resistance.

  RESULTS : Vitamin D was significantly correlated to insulin resistance and beta cell function in their multiethnic sample. Concluded low vitamin D levels play a significant role in the pathogenesis of type 2 diabetes.

- Pittas et al (48)

  RESULTS : Insufficient vitamin D and calcium appears to hinder glycemic control and that supplementing both nutrients may be necessary to optimize glucose metabolism.

- Nurses Health Study (50)

  - Observational study.

  - 83,779 women > 20 years were studied.

  RESULTS : Found an increased risk of type 2 diabetes in those with low vitamin D status. A combined daily intake of > 800 IU of vitamin D and 1,000 mg of calcium reduced the risk of type 2 diabetes by 33%.

- The National Health and Nutrition Examination Survey (NHANES) III study (51)

  Study period 1988 — 1994

  RESULTS : There is a strong inverse association between low levels of 25(OH)D and diabetes prevalence. Low vitamin D levels have also been shown to be predictive of the future development of type 2 diabetes.

The following are other important studies proving the association of vitamin D deficiency and type 2 diabetes :

Study design	Subjects included	Main outcome
Cohort (Mini-Finland Health Survey) (52)	4097 individuals followed-up for 17 years.	The highest *versus* the lowest serum 25OHD: RR = 0.70; 95% CI = 0.42–1.16); *p* for trend = 0.07).
Cohort (Tromsø Study) (53)	4157 non-smokers and 1962 smokers followed-up for 11 years.	Baseline serum 25OHD was inversely associated with type 2 diabetes.
Cohort (Nurses' Health Study) (54)	83,779 women followed-up for 20 years.	The highest *versus* the lowest category of vitamin D intake from supplements: RR = 0.87; 95% CI = 0.75–1.00; *p* for trend = 0.004).
Nested case-control (55)	412 cases and 986 controls.	The highest *versus* the lowest quartiles of serum 25OHD: OR = 0.28 (95% CI = 0.10–0.81) in men and OR = 1.14 (95% CI = 0.60–2.17) in women.
Meta-analysis (56)	Polled data from 2 cohorts studies with 8627 individuals aged 40–79 years.	The highest *versus* the lowest serum 25OHD: RR = 0.66; 95% CI = 0.50–0.87.
Cohort (Framingham Study) (57)	3066 (1402 men and 1664 women) followed-up for 7 years.	A higher 25OHD serum levels is associated with decreased risk of type 2 diabetes.
Nested case-control (58)	608 cases and 559 controls.	The highest *versus* the lowest serum 25OHD quartile: OR = 0.52; 95% CI = 0.33–0.83.
Cross-sectional (59)	210 individual aged more than 40.	Vitamin D deficiency was more common in diabetic compared to control.
Cross-sectional (60)	668 individuals aged 70–74 years.	Serum 25OHD < 50 nmol/L doubled the risk of newly diagnosed type 2 diabetes.
Cohort (AusDiab study) (61)	5200 individuals; mean age 51 years.	Each 25 nmol/L increment in serum 25OHD was associated with a 24% reduced risk of type 2 diabetes (OR = 0.76; 95% CI = 0.63–0.92).
Cross-sectional (62)	2465 subjects.	Serum 25OHD ≥ 80 nmol/L *versus* ≤37 nmol/L in Caucasians: OR = 0.5; 95% CI = 0.1–0.7.
Systematic review of 7 observational cohort studies. (63)	238,424 individuals aged 30–75 years.	Vitamin D intake >500 *versus* <200 UI: risk of type 2 diabetes 13% lower. Serum 25OHD level (>25 ng/mL *versus* <14 ng/mL): risk of type 2 diabetes 43% lower.

VITAMIN D STATUS IN INDIA:

Despite India being a tropical country with plenty of sunshine vitamin D deficiency is still an epidemic in India due to several factors :

1) Urbanisation and changes in food style contribute to low intake of both calcium and vitamin D.

2) Rich fibre diet contains phosphates and phytates that impairs vitamin D absorption and can increase its requirement and deplete vit D stores (64).

3) Heritability of vitamin D binding protein determines the increment in serum 25 hydroxy vit-D response to treatment(65).

4) Genetic factors like having increased 25(OH)D-24 Hydroxylase enzyme which degrades 25(OH)D to inactive metabolites, also have a role (66).

5) The duration of time spent indoor is increasing in urban population due to the rise in indoor recreation facilities which prevents adequate sun exposure.

6) Air pollution due to increased automobile usage hamper the UV rays from reaching the skin (67).

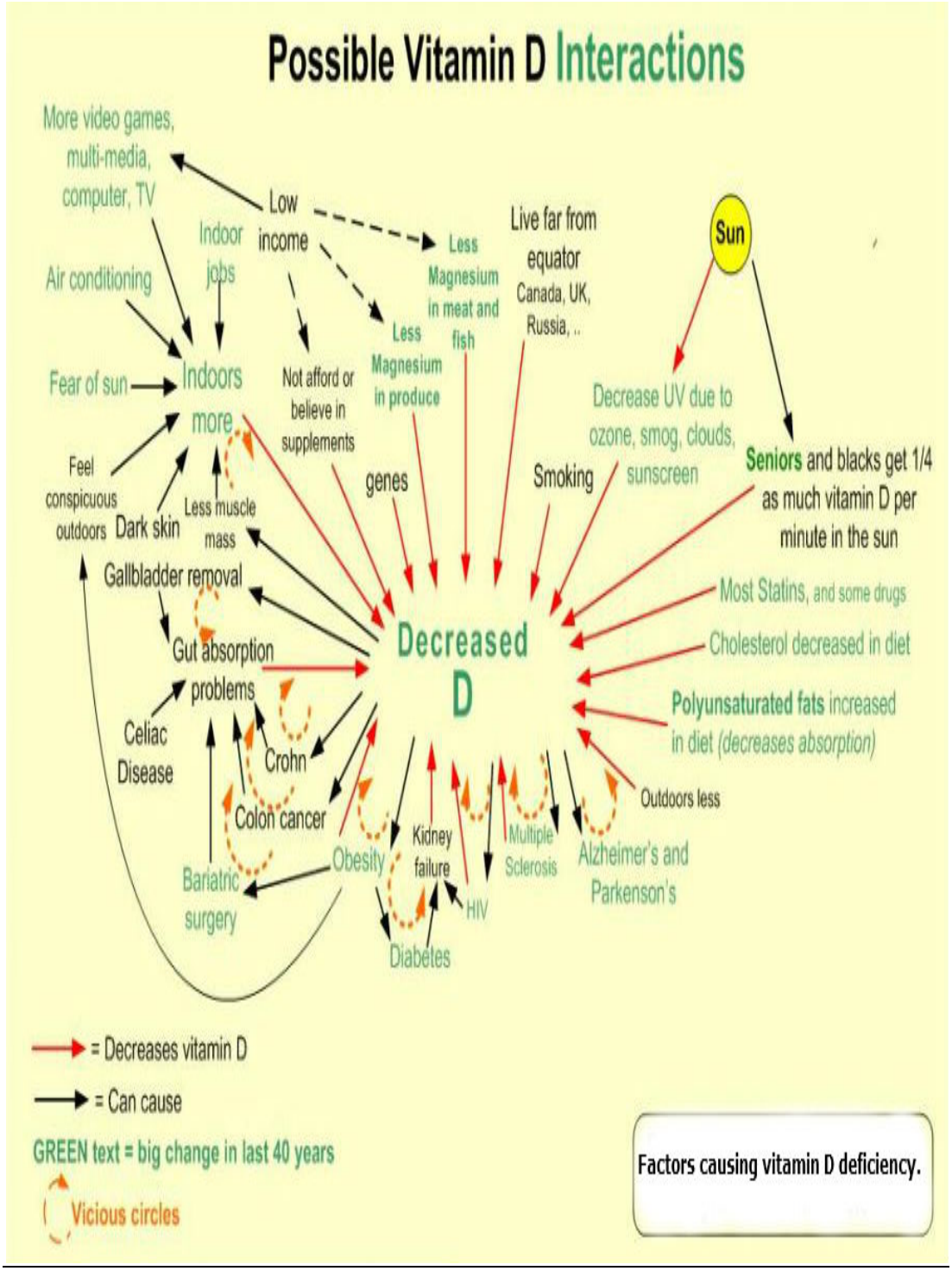
7) Repeated pregnancies in dietary deficient young females aggravates vitamin D deficiency in both mother and baby.

8) Traditional religious superstitions in muslim women like wearing ‘pardah’ is still prevalent making them vulnerable to be vitamin D deficient.

The following are suggested risk factors for people to be vit D deficient.

Table. **Risk Factors for Vitamin D Deficiency**

Advanced age
Institutionalized or home-bound
Use of sunscreen with sun protection factor >15
Heavily pigmented skin
Air pollution
Prolonged, exclusive breastfeeding
Northern latitudes
Smoking
Obesity
Malabsorption syndromes
Renal or liver disease
Antiepileptic or HIV medications


Possible Vitamin D Interactions
More video games, multi-media, computer, TV
Air conditioning
Indoor jobs
Low income
Less Magnesium in meat and fish
Live far from equator Canada, UK, Russia, ..
Sun
Fear of sun
Indoors more
Not afford or believe in supplements
Less Magnesium in produce
Decrease UV due to ozone, smog, clouds, sunscreen
Seniors and blacks get 1/4 as much vitamin D per minute in the sun
Feel conspicuous outdoors
Dark skin
Less muscle mass
genes
Smoking
Gallbladder removal
Most Statins, and some drugs
Gut absorption problems
Decreased D
Cholesterol decreased in diet
Polyunsaturated fats increased in diet (decreases absorption)
Celiac Disease
Crohn
Outdoors less
Colon cancer
Kidney failure
Multiple Sclerosis
Alzheimer's and Parkenson's
Bariatric surgery
Obesity
HIV
Diabetes
= Decreases vitamin D
= Can cause
GREEN text = big change in last 40 years
Vicious circles
Factors causing vitamin D deficiency.

INDICATIONS FOR VITAMIN D TESTING: (68)

1) Patients diagnosed with any of the vitamin D related diseases like rheumatoid arthritis, myocardial infarction and stroke especially in the young, diabetes mellitus with poor glycemic control, cancer, pulmonary tuberculosis especially drug resistant, SLE, epilepsy and others..)

2) Patients with bone diseases like osteoporosis, rickets/osteomalacia.

3) Patients with chronic nonspecific musculoskeletal pain.

4) Signs of mental depression and asthenia

5) Patients with gastrointestinal disease and who have had intestinal resection/cholecystectomy.

6) Elderly people

7) Obese people with BMI > 25.

8) Exclusively breastfed infants.

9) Individuals taking vitamin D supplements > 50 mcg/day

10) Individuals who live above 42° north latitude where zenith angle is low.

11) Individuals with dark complexions or who do not regularly receive 20 minutes of direct sunlight each day especially software IT professionals.

METHODS FOR TESTING VITAMIN D:

25(OH) vitamin D can be measured as individual components (D2, D3) or as a whole, but not all assays that are available have the same sensitivity to 25(OH) vitamin D2 and 25(OH) vitamin D3. Some immunoassays only detect one type of 25(OH) vitamin D and others do not fully detect the entire amount of each form of 25(OH) vitamin D. No matter what method is used, the most important value is the total serum 25(OH) vitamin D value because it represents the total amount of vitamin D (both D2 and D3) that is available and circulating. It is also the same measure as used in the various health-based reference values. This ensures that the patients receive the most reliable result to determine their vitamin D status, regardless of level and type of supplementation (69).

Vitamin D deficiency can be measured (70) by using :

1. Immuno Assay or Protein binding Assay

   - report total vitamin D

2. Chemical Assay- can report vitamin D2 and D3.

Classification of vitamin D assay :

Assay	**Technology**	**Advantages**	**Disadvantages**
Immuno Assay	RIA	Specific, sensitive, highly reproducible, cost effective	Manual Assay
	CLIA ECL	Sensitive, Auotmated	Reagent issues, Problems with reproducibility
Chemical Assay	HPLC-UV, GC-MS, LC-MS	Specific, sensitive, highly reproducible	Very costly, for research purposes only

Recommendations on vitamin D assay :

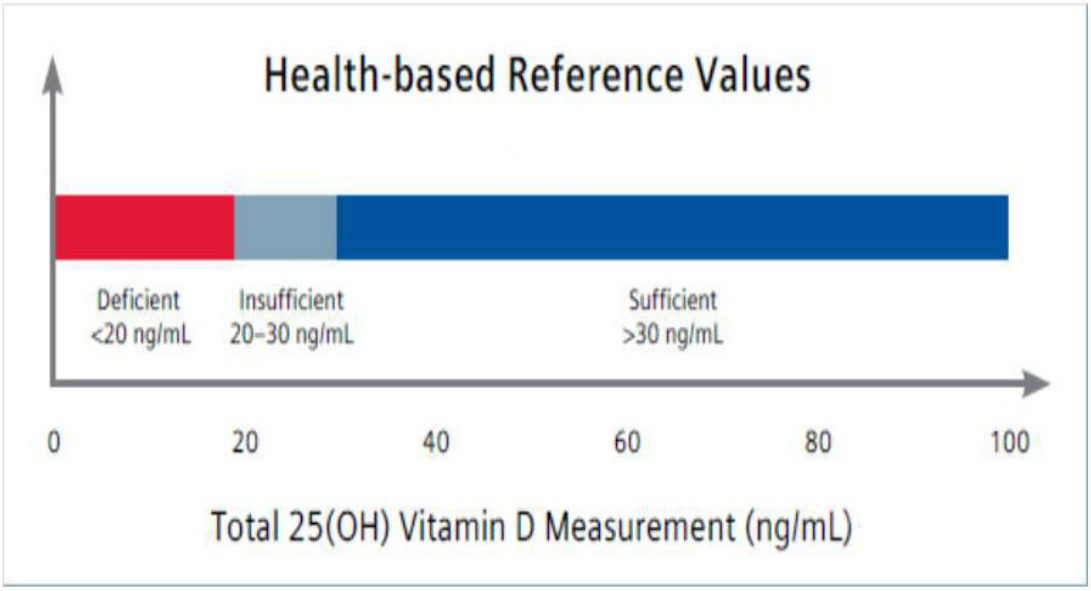
1) Use an assay that measures both vitamin D2 and D3. Serum is the ideal sample type

2) Report the total vitamin D in ng/ml.

Reports should include total 25 (OH) D health based reference values & not population based reference ranges

REFERENCE RANGES FOR ASSESSING VIT-D STATUS :

Although there is no well well formed universal consensus on vitamin D reference values, many health care professionals believe that health based reference values are most preferable. Accordingly the following value ranges are used (71):



MANAGEMENT OF VITAMIN D DEFICIENCY:

The following are the recommended dietary allowances for vitamin D with respect to specific age groups as put forth by the 'Food and Nutrition Board'(72):

Table 2: Recommended Dietary Allowances (RDAs) for Vitamin D [1]

Age	Male	Female	Pregnancy	Lactation
0–12 months*	400 IU (10 mcg)	400 IU (10 mcg)		
1–13 years	600 IU (15 mcg)	600 IU (15 mcg)		
14–18 years	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)
19–50 years	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)
51–70 years	600 IU (15 mcg)	600 IU (15 mcg)		
>70 years	800 IU (20 mcg)	800 IU (20 mcg)		

* Adequate Intake (AI)

Three options exist for the treatment of vitamin D deficiency: sunlight, artificial UVB light or vit D supplements (73).

Sunlight :

Adequate exposure to sunlight especially between 10 am and 3 pm produces vit D in the skin that last twice as long in the blood compared

with ingested vitamin D. If sun exposure produces slight pinkness the amount of vit D produced is equivalent to ingesting 10,000-25000 IU(74).

Diet and UVB lamps:

Since food is a poor source of vitamin D with the exception of coldwater ocean fish, dietary methods only supplement other treatment methods and cannot be used alone. Foods rich in vitamin D has been already mentioned previously.

A study from Sweden comparing full body irradiation with artificial UVB lamps (3 times/week for 6 weeks) found UVB therapy more efficacious in rising vit D concentrations (75).

Vitamin D supplements:

The treatment of choice for vitamin D deficiency is Vitamin D3 (Cholecalciferol). Each 1000 IU of vitamin D3 in addition to a person's food supply will rise the level of 25(OH)D by 10 ng/ml when it is constantly supplemented over a period of 3 months. Therefore, a patient with pretreatment level of 10 ng / ml will usually require 3000 IU/ day for many months to achieve a level of 40 ng / ml and 4000 IU / day to achieve a level of 50 ng /ml – in the absence of UVB exposure (76).

The recommended doses are stated below and it is to be noted that these doses are in addition to what patient is ingesting at baseline. Patients receiving > 2000 IU of vit D daily should have their serum level tested once in every 12 weeks.

(77)

Recommendations for Treatment of Vitamin D Deficiency or Insufficiency

Serum 25(OH)D Level (ng/mL)	Vitamin D3 Supplementation Dose (IU)	Frequency of Vitamin D Supplementation
20-30	1,000-2,000	daily
12-20	2,000	daily
< 12 (asymptomatic)	4,000-5,000	daily
< 12 (symptomatic)	30,000	weekly

It should also be noted that obese individuals may need higher doses because of the fact that vit D gets sequestered in adipose tissue.

Vitamin D toxicity:

Vitamin D toxicity (usually asymptomatic hypercalcemia) is exceedingly rare (78). True toxicity is secondary to the unbridled effects of hypercalcemia. In order to produce hypercalcemia one has to take more than 10,000 IU / day for many months or even years and when serum 25(OH)D exceeds 150 ng/ml.

Given the multitude of actions and uniqueness of vitamin D makes most educated patients regulate their own tissue levels of this vitamin, either through UVB exposure or pharmacological supplements. Given the attitudes in medicine that any substance with the word ‘Vitamin’ in it (79), the public and not the medical profession may be the first to enter the vitamin D era.

HBA1C :

HbA1c refers to ‘glycated hemoglobin’ that identifies average blood glucose concentration over a period of time (2-3 months). Glucose molecules in the blood normally becomes attached to haemoglobin- that is the haemoglobin becomes glycosylated. As a persons blood glucose becomes higher more of his haemoglobin becomes glycosylated. The

glucose remains attached to the haemoglobin for the life of the RBC's which is around 2-3 months (80).

American Diabetes Association (ADA) currently recommends an HbA1c of 6.5% as a cut off for diagnosing diabetes (81).

ADA Recommendations (82):

A1c :

1) Do HbA1C test atleast two times a year in diabetics who achieve glycemic targets.
2) Do HbA1C test quarterly in diabetics who have a recent change in therapy or those who do not achieve glycemic targets.
3) Use of point of care testing for HbA1c allow for timely decisions on therapy changes when needed.

Glycemic targets in adults:

HbA1c when lowered to 7% or below decreases both microvascular and neuropathic complications of both types of diabetes. Therefore the target HbA1c goal in nonpregnant adults is in general <7%.

The following HbA1c reference ranges depicts the appropriate glycemic status in diabetic patients (83).

HbA1c	Normal/abnormal
4.0 - 6.0%	Normal for those without diabetes
6.1 – 7.0%	Target range for those with diabetes
7.1 – 8.0%	High
8.1 – 9.0%	Too high
Greater than 9.1%	Very high

Currently HbA1c values are used as,

1) Measure of glycemic control in diabetics.

2) Indicator of risk level for diabetic complications.

3) Measure of quality of diabetes care.

It is currently considered the best index for metabolic control in diabetes (84).

The three main HbA1c testing methods that most clinical laboratories uses are (85):

1) High pressure Liquid chromatography (HPLC) based assay

2) Immunoassay – Based on antibody.

3) Enzymatic assays.

MATERIALS AND METHODS

Patients included in the study were recruited from the Govt. Mohan Kumaramangalam Medical College & Hospital.

The study period :

From October 2010 to September 2012.

Type of study : Observational study.

Inclusion criteria:

100 diabetic patients attending diabetic op and admitted in ward formed the study group.

Exclusion criteria

1. Patients with established chronic kidney disease.
2. Patients with established chronic liver disease.
3. Patients on glucocorticoid therapy for any cause.
4. Patient on antiseizure medications.

INVESTIGATIONS:

Duration of diabetes mellitus, medications patients currently on, other associated problems like SHT, Coronary artery heart disease, etc was sought from patients history. Body Mass Index (BMI) is calculated using standard formula.

- Blood samples will be collected for total vitamin D and HbA1c levels estimation and was sent to thyrocare labs. Vit D levels were estimated using chemiluminescent immune assay (C.L.I.A).

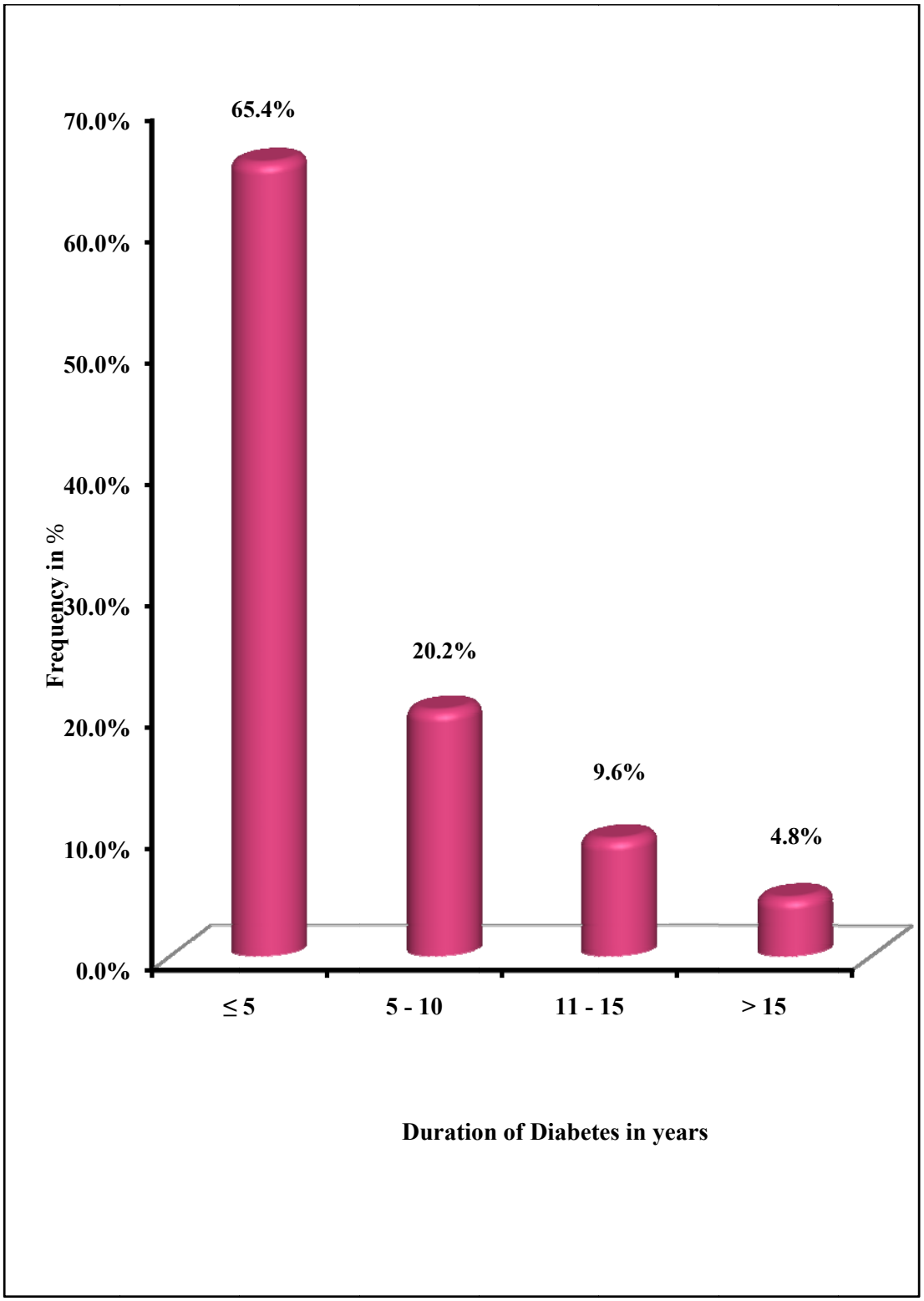
HbA1c value is measured using H.P.L.C using TOSOH G8.

Correlation between Glycemic control as assessed by HbA1c value and vit-D levels was analysed. SSPS software was used for statistical analysis.

DURATION OF DIABETES :

Average duration of diabetes in years were shown below. Most patients in the study have the disease duration less than 5 years.

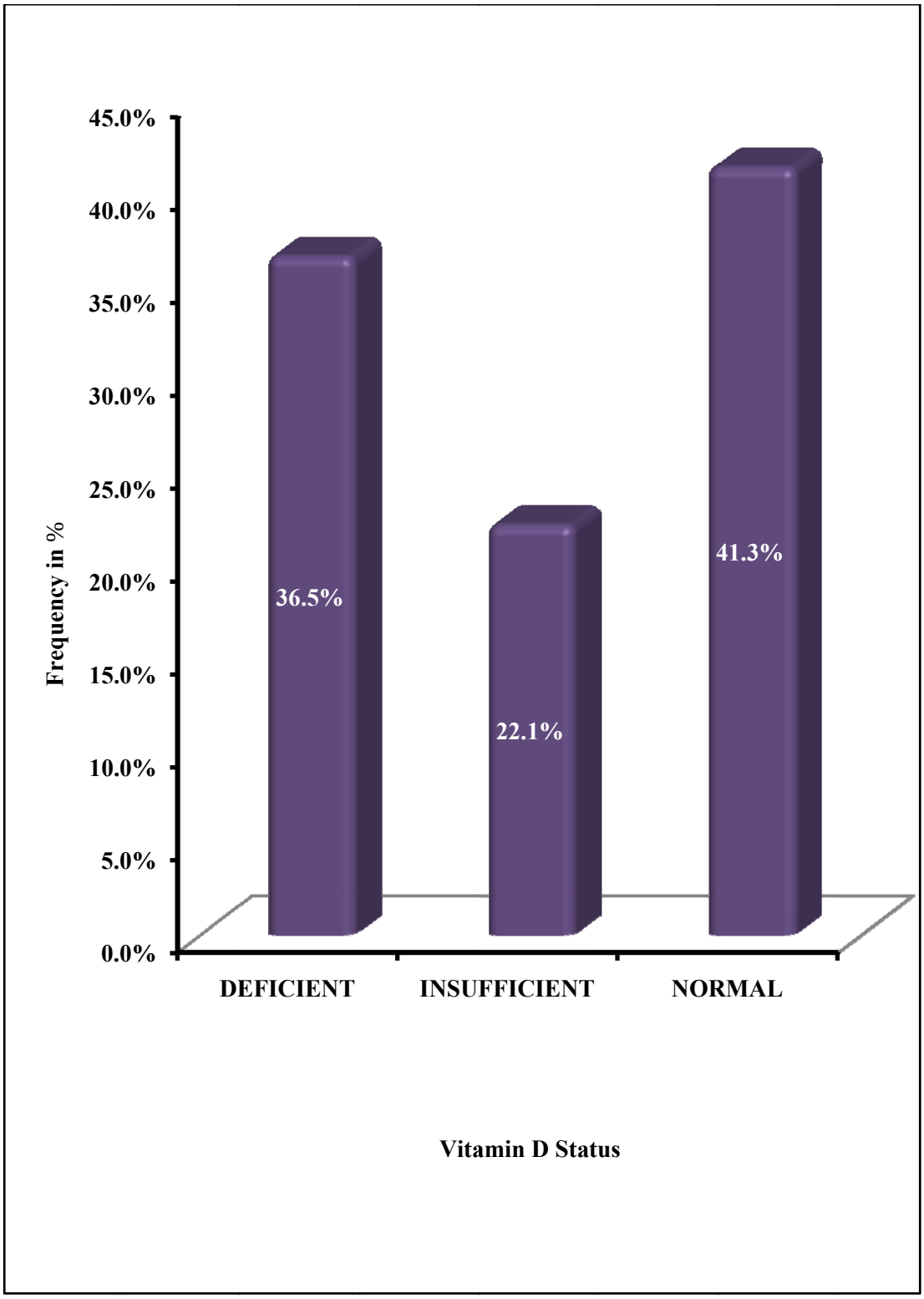
Duration of Diabetes In years	Number	Percentage %
≤ 5	68	65.4%
5 - 10	21	20.2%
11 - 15	10	9.6%
> 15	5	4.8%
Total	104	


70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
Frequency in %
65.4%
20.2%
9.6%
4.8%
≤ 5
5 - 10
11 - 15
> 15
Duration of Diabetes in years

VIT- D STATUS :

The vitamin D status of the patients were assessed based on the health based reference values discussed previously. (Sufficient - >30 ng/ml, Insufficient 20-30 ng/ml, Deficient < 20 ng/ml.

VITAMIN D STATUS	NUMBER	PERCENTAGE
DEFICIENT	37	36.5%
INSUFFICIENT	23	22.1%
NORMAL	44	41.3%
Total	104	


45.0%
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Frequency in %
36.5%
22.1%
41.3%
DEFICIENT
INSUFFICIENT
NORMAL
Vitamin D Status

HBA1C LEVELS OF PATIENTS :

The HbA1C level of patients were rated as target range (6-7 %) high (7-8%) too high (8-9%) and very high (> 9%).

HbA1C		No	%
6 - 7	**Target range**	6	5.8%
7 - 8	**High**	29	27.9%
8 - 9	**Too High**	15	14.4%
> 9	**Very High**	54	51.9%
Total		104	